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FACTORS AFFECTING THE FRUITING
OF OPHIOPOLUS GRAMINIS SACC.


John D. Gilpatrick

Department of Plant Science

University of Alberta

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FACULTY OF AGRICULTURE

The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance a thesis on "Factors affecting the fruiting of Ophiobolus graminis Sacc.", submitted by J. D. Gilpatrick, B.Sc. (Agr.) in partial fulfilment of the requirements for the degree of Master of Science.

FACTORS AFFECTING THE FRUITING
OF OPHIOBOLUS GRAMINIS SACC.

J. D. Gilpatrick
Department of Plant Science

A THESIS
submitted to the University of Alberta
in partial fulfilment of the
requirements for the degree of
MASTER OF SCIENCE

Edmonton, Alberta
September, 1948

thesis
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FACTORS AFFECTING THE FRUITING OF
OPHIOBOLUS GRAMINIS SACC.

J. D. Gilpatrick

INTRODUCTION

Ophiobolus graminis Sacc., the cause of Take-all of wheat and other members of the Gramineae is a destructive parasite in many of the wheat-growing areas of the world. Although several aspects of this fungus have been carefully studied its fruiting habits until recently have been given little more than passing attention in the literature. Since this phase of the organism's activity has been neglected, the studies reported here were undertaken.

O. graminis fruits by the production of but one spore stage. Fruiting bodies called perithecia bearing the spores, when formed on the host, are found near the soil level. These bodies however are not always formed in nature and are rarely found on infected wheat plants in Alberta fields.

Their occurrence in this province is apparently much less common than in certain other wheat-growing regions such as those of the eastern United States and the British Isles. That sporulation occurs only rarely in pure culture has been reported by Davis (16), Davies (14), Russell (72), Hynes (40), and Garrett (25) and has been confirmed by observations made over several years at this laboratory.

A better knowledge of the factors affecting sporulation of O. graminis would be useful for several reasons. Firstly, any method of inducing the organism to fruit on diseased tissue would facilitate isolation which is ordinarily difficult if ripe perithecia are not present. Secondly, isolates could be more readily and conclusively indentified. Thirdly, a sufficient supply of spores would be available for genetical studies. Fourthly, O. graminis exists as many different strains in nature and several of these may be isolated from a single area. Probably these strains differing in pathogenic capabilities on the host and cultural characters on agar arise through the sexual process. Thus a simple method of inducing fruiting is necessary for studies of physiologic specialization of this organism. Fifthly, although Garrett (23) has concluded that it is very unlikely that spores can play any part in the dispersal and survival of this organism under field conditions, there seems to be need for further studies on the relationship of ascospore

the production to epiphytology of "Take-all". A plentiful supply of spores produced at will would be required for such studies. Finally, a knowledge of the factors promoting fruiting of this fungus, which sporulates erratically, would probably contribute to the understanding of the sporulation of fungi in general.

The purpose of the work reported here was to contribute to the understanding of the mechanism of sporulation by studying in pure culture and on the host the effect of various factors on this process. Furthermore, attempts were made to develop a simple repeatable technique for the production of mature perithecia.

Several factors have been suggested as playing a part in the fruiting process of O. graminis. None as yet has been demonstrated as being critical. Physical, nutritional, and biological factors affect the sporulation of fungi in general. Of the physical factors, light, soil moisture, and humidity are important. Nutritional aspects include both the quality and quantity of food. The interaction of associated microorganisms with O. graminis may be an important biological factor.

Biological and nutritional factors are stressed in the present study. Although physical conditions receive less direct attention they are always kept in view when other factors are under consideration and are maintained at levels thought likely to be optimum for the sporulation of the organism.

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DESCRIPTION OF OPHIOBOLUS GRAMINIS

The life cycle of many fungi consists typically of a mycelial or vegetative stage and two reproductive stages - sexual and asexual. Although conidial or asexual spore stages of O. graminis have been reported, their occurrence has never been confirmed. The sexual stage is characterized by the production of flask-shaped perithecia in which are borne banana-shaped sacs or asci, each containing 8 ascospores. The spores are long, slender, hyaline, septate, and lie parallel in the ascus. These structures are illustrated in Figures 1 and 2.

Measurements given by various workers for the length of ascospores of this organism vary from 60 to 118 microns (72). Ascospore lengths were measured from time to time in the present study. In one instance spores from perithecia formed on wheat plants, grown under ordinary conditions in the greenhouse, varied from 60 - 111 microns in length. The mean length of 100 spores was 92.9 ± 19.2 . These ascospore lengths seem to agree with those previously reported in the literature.



Figure 1

Perithecium and asci of Ophiobolus graminis
Sacc. formed in pure culture -
after F. R. Davies (14)



Figure 2

Asci and ascospores of Ophiobolus graminis from a perithecium formed on a wheat seedling.

Note that the septate ascospores lie parallel in the ascus.



Figure 2

For the purpose of this study, the following data were collected from the field. The data were collected in the form of a questionnaire. The questionnaire was distributed to the respondents in the form of a questionnaire. The questionnaire was distributed to the respondents in the form of a questionnaire. The questionnaire was distributed to the respondents in the form of a questionnaire.



Figure 3

Wheat seedling infected with Ophiobolus graminis by the flask method. Note the unusually long, protruding beaks of the perithecia of the organism curving upward toward the light.

O. graminis attacks the basal parts of the host from the roots to several inches above the crown. A plate of brown mycelium forms between the outer and inner leaf sheaths and between the inner leaf sheath and the culm. It is from these plates of mycelia that the perithecia arise. When they are mature their curved beaks may sometimes be seen protruding through the outer leaf sheath (Figure 3).

In the class Ascomycetes, of which O. graminis is a member, the development of asci and perithecia seem to be two separate processes, the ascus resulting from a sexual process and the perithecium arising from vegetative hyphae. Both processes might be dependent on the same stimulatory effect or different effects. If there are different mechanisms then conceivably a perithecium or ascus might be induced to form without the other structure being present. Although naked asci have never been reported, perithecia-like bodies which bear no asci are often observed in artificial culture. These structures are usually regarded as immature perithecia.

Jones (41) has described cytological details of the development of mature perithecia of O. graminis*. He found that ascospore production is initiated by the conjugation of two or more vegetative cells. From such conjugated cells ascogenous hyphae arise with binucleate cells. This process occurs within a fairly well developed perithecium. Ascus development appears to take place from any binucleate cell of an ascogenous hyphae. Nuclear fusion occurs in the young ascus. This is followed by three divisions of the nuclei one of which is a reduction division. The resulting 8 nuclei are then walled off one to a unit and each unit eventually develops into an ascospore.

Thus according to Jones' description the initiation of perithecial formation is not dependent on the conjugation process. Therefore it is possible to conceive stimulation of perithecia without further stimulation of ascus formation. In the light of available information this conception is purely speculative.

* Since Jones' work was reported, Miss Turner (Trans. Brit. Mycol. Soc. 24:269-281. 1940) has demonstrated that certain isolates of O. graminis from oats are distinctly different from those of wheat and she regards them as belonging to a new variety avenae. It is possible that the strains that Jones worked with belonged to this variety.

The first part of the report is devoted to a general survey of the situation in the country. It is followed by a detailed account of the work done during the year. The report concludes with a summary of the results and a list of recommendations.

The work done during the year has been of a very satisfactory nature. It has been carried out in accordance with the programme of work approved by the Council of the League of Nations. The results of the work are set out in the following table:

1. General survey of the situation in the country.	2. Detailed account of the work done during the year.
3. Summary of the results.	4. List of recommendations.

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3. Summary of the results.	4. List of recommendations.

The results of the work are of a very satisfactory nature. It has been carried out in accordance with the programme of work approved by the Council of the League of Nations. The results of the work are set out in the following table:

In these studies two isolates of the organism designated as S_1 and S_2 were characterized by the formation of small, black spermagonia-like bodies in the presence of certain growth factors. These were usually covered with a tuft of white mycelium. When formed in the centre of a culture they were roundish in shape (Figure 4), but when formed at the junction of glass and agar they were ovoid and possessed long, beak-like appendages. These bodies produced no asci or ascospores but at times were observed to exude tiny structures resembling spores the exact nature of which was not determined. Several strains including S_1 formed similar bodies which were naked and never covered with a tuft of mycelium. Nor were they ever observed to exude spore-like bodies.

Jones (41) reported that O. graminis forms spermagonia and spermatia (male sex elements) but he considered them to be functionless. At maturity the spermagonia were spherical or ovoid in shape. They produced small cells of narrow diameter, blunt at one end and narrowing to a point at the other extremity. These bodies measured from 5 - 7 microns by 1 - 1.5 microns, were distinctly curved, and possessed a prominent nucleus. These same structures have not been reported by other workers.

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. The text also mentions the need for regular audits to ensure the integrity of the financial data.

In the second section, the author outlines the various methods used for data collection and analysis. This includes both manual and automated processes. The importance of data security is also highlighted, with a focus on protecting sensitive information from unauthorized access.

The third part of the document provides a detailed overview of the current market conditions. It discusses the impact of recent economic events and offers insights into future trends. The author also includes a list of key indicators that are being monitored closely.

Finally, the document concludes with a summary of the findings and a list of recommendations. It stresses the need for continued vigilance and adaptability in the face of a rapidly changing environment.

The following table provides a summary of the key data points discussed in the report. It includes values for various metrics and a comparison to previous periods.

Metric	Current Value	Previous Period
Revenue Growth	12.5%	10.2%
Profit Margin	18.3%	17.1%
Customer Satisfaction	85%	82%
Operational Efficiency	92%	90%

The data indicates a positive trend across most key areas, suggesting that the current strategies are effective. However, there are still areas for improvement, particularly in operational efficiency and customer retention.

The author concludes by reiterating the importance of staying informed and proactive in managing the business. Regular communication and collaboration are essential for long-term success.

The small spore-like bodies observed in these studies may have been of the same nature as those observed by Jones. However they were much shorter, possessed no prominent nucleus, and did not appear constricted at one end. However, some of them were definitely curved. The black-bodies produced in these tests which bore tiny spores possessed long beaks or appendages under certain conditions. Jones' spermagonia were not so characterized. Because of the nature of the spores produced by these bodies in these studies they are not perithecia. However it seems logical to assume that they may be spermagonia similar to those produced by other members of the Ascomycetes closely related to O. graminis.

It is possible that the non-spore-forming black bodies produced in these tests were immature perithecia. The proper conditions may not have been provided for conjugation to take place or for the subsequent development of mature ascospores.

The first of these is the fact that the
 Government has not yet decided upon a
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 Government has expressed its
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 it will actually veto them or
 whether it will allow them to
 pass. This uncertainty has
 caused much confusion among
 the people, and it is
 therefore necessary that the
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 its policy as soon as possible.

It is also necessary that the
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 it will allow them to pass.

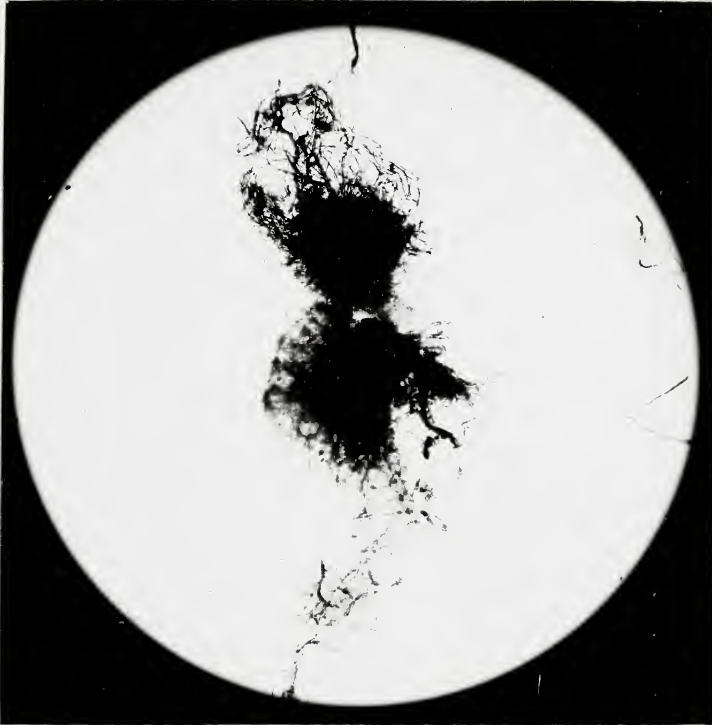


Figure 4

Spermagonia-like bodies produced by strains S_1 and S_2
of Ophiobolus graminis in pure culture.

SEXUALITY OF O. GRAMINIS

Introduction

Fungi which require the intermingling of thalli of different strains before sexual reproductive bodies will form are spoken of as being heterothallic. Those fungi requiring no such interaction of strains are described as homothallic.

O. graminis is known to have many different strains with varying pathogenic capabilities on the host and different morphological characteristics in pure culture. This physiologic specialization has been observed at this laboratory and by workers elsewhere (25, 60). Russell (73) has discussed the subject in a separate paper.

Because O. graminis exists as different strains the question arises as to whether the organism is homo- or heterothallic, and as to whether different strains differ in their sporulating abilities. These problems were investigated in the following studies in an attempt to determine the role that physiologic specialization may play in the sporulation of O. graminis.

THE

REPORT

The first section of the report is devoted to a general survey of the situation in the country. It is followed by a detailed account of the work done during the year. The third section contains a list of the names of the persons who have been employed during the year. The fourth section contains a list of the names of the persons who have been employed during the year.

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Studies with Mixed Strains

Literature Review

The sexuality of O. graminis has been investigated by several workers. Kirby (46) reported that all of his New York strains of this organism were heterothallic. He stated that in order to get sporulation in pure culture it was necessary to grow + and - strains together. This same worker, however, later found other isolates which would produce perithecia without the stimulus of a second strain (47).

Davis (16) found no evidence of heterothallism when a single spore isolate originating from one of Kirby's "heterothallic" New York strains was grown in agar culture. This isolate sporulated as well when grown alone as it did when in combination with two other strains. The latter failed to sporulate under any conditions of the environment. Davis felt that this lack of vigor was the result of long culture in an artificial medium. He noticed an interaction along the line where two colonies of the same or of different strains approached each other. This was characterized by a darkening of the mycelium of all strains and the formation of perithecia by the New York strain.

Davis concluded that although his New York strain was homothallic that nutrition or the products of metabolism may act as a stimulant for sporulation whereas otherwise the organism would not fruit. He suggested that this postulation might explain Kirby's results.

Both Davies (14) and White (84) found evidence in favor of homothallism but could offer no support to the theory of a nutritive stimulus. Davies reported that monosporous cultures of the organism produced perithecia abundantly in Petri plates under certain conditions. Growth of four different strains in combination did not induce fruiting in one case although two of the strains grown separately on diseased wheat seedlings produced perithecia. White found that monosporous isolates of O. graminis formed perithecia when grown on roots of wheat plants in pure culture on nutrient agar in large tubes. He also found that 8 monosporous isolates grown on potato dextrose agar sporulated no more readily in combination than alone.

Experimental Studies

With evidence for and against a nutritive stimulus for reproduction and with the absence of a satisfactory explanation of Kirby's results, apparently there is a need for further investigations of this problem. Consequently

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two experiments involving mixed strains were conducted in these studies: one on agar in Petri plates; the other on wheat seedlings in pots.

1. Petri plate studies

Various strains of O. graminis were grown singly and in various combinations on potato dextrose agar in Petri plates which were incubated at 15° - 20°C in diffuse natural light conditions in the greenhouse. Bits of mycelium of the strains were transferred from agar slants to the surface of the medium in the plate. Two plantings spaced about 50 mms. apart were made in each plate. The cultures were observed for 8 weeks before discarding. The strains used and the results of the experiment are presented in Table 1.

No perithecia were formed in any of the plates. The rough type of growth produced by strains S₁ and S₂ is characteristic of these strains on various media. (See Figure 5). The black bodies resembling spermatogonia were formed over the plates but most abundantly where two colonies approached each other or at the point of contact of agar and glass.

TABLE I

COLONY INTERACTION OF DIFFERENT STRAINS OF O. GRAMINIS

Strains in combination	Number of perithecia produced	Antagonism between colonies
8; 8	0	0
8; III-2	0	++
8; III-14	0	++
8; III-17	0	+
8; S ₁	0	++
8; S ₂	0*	+
S ₁ ; S ₁	0*	+
S ₁ ; III-2	0*	++
S ₁ ; S ₂	0*	++
S ₂ ; S ₂	0*	+
III-2; III-2	0	0
III-2; S ₂	0*	++

* = Black tufts resembling immature spermatogonia produced by strains S₁ and S₂.

0 = No perithecia or no antagonism.

+ = Slight antagonism.

++ = Moderate antagonism with definite clean zone between colonies.

I - 10

TABLE I - SUMMARY OF RESULTS

Year	Number of cases	Number of deaths	Number of recoveries
1950	10	0	10
1951	15	2	13
1952	20	3	17
1953	25	4	21
1954	30	5	25
1955	35	6	29
1956	40	7	33
1957	45	8	37
1958	50	9	41
1959	55	10	45
1960	60	11	49
1961	65	12	53
1962	70	13	57
1963	75	14	61
1964	80	15	65
1965	85	16	69
1966	90	17	73
1967	95	18	77
1968	100	19	81
1969	105	20	85
1970	110	21	89
1971	115	22	93
1972	120	23	97
1973	125	24	101
1974	130	25	105
1975	135	26	109
1976	140	27	113
1977	145	28	117
1978	150	29	121
1979	155	30	125
1980	160	31	129
1981	165	32	133
1982	170	33	137
1983	175	34	141
1984	180	35	145
1985	185	36	149
1986	190	37	153
1987	195	38	157
1988	200	39	161
1989	205	40	165
1990	210	41	169
1991	215	42	173
1992	220	43	177
1993	225	44	181
1994	230	45	185
1995	235	46	189
1996	240	47	193
1997	245	48	197
1998	250	49	201
1999	255	50	205
2000	260	51	209
2001	265	52	213
2002	270	53	217
2003	275	54	221
2004	280	55	225
2005	285	56	229
2006	290	57	233
2007	295	58	237
2008	300	59	241
2009	305	60	245
2010	310	61	249
2011	315	62	253
2012	320	63	257
2013	325	64	261
2014	330	65	265
2015	335	66	269
2016	340	67	273
2017	345	68	277
2018	350	69	281
2019	355	70	285
2020	360	71	289
2021	365	72	293
2022	370	73	297
2023	375	74	301
2024	380	75	305
2025	385	76	309
2026	390	77	313
2027	395	78	317
2028	400	79	321
2029	405	80	325
2030	410	81	329

1. The number of cases reported in this table is based on the data received from the health authorities of the various countries. The number of deaths is based on the data received from the same authorities. The number of recoveries is based on the data received from the same authorities.

2. The number of cases reported in this table is based on the data received from the health authorities of the various countries. The number of deaths is based on the data received from the same authorities. The number of recoveries is based on the data received from the same authorities.

3. The number of cases reported in this table is based on the data received from the health authorities of the various countries. The number of deaths is based on the data received from the same authorities. The number of recoveries is based on the data received from the same authorities.

4. The number of cases reported in this table is based on the data received from the health authorities of the various countries. The number of deaths is based on the data received from the same authorities. The number of recoveries is based on the data received from the same authorities.

Antagonistic reactions occurred where two colonies of different strains approached each other; between colonies of the same strain there was only slight or no inhibition (See Figure 5). A ruffled type of growth was observed along the line of approach of antagonistic colonies.

2. Pot studies

In a similar test carried out in seven inch pots in the greenhouse, highly pathogenic strain 8 was used singly and in combination with 4 other less pathogenic strains as inoculum for infecting wheat. These strains were grown separately on a soil medium containing 10% corn meal by weight. The single strain inocula thus produced were added to the pots of sterilized soil at seed level. When inocula of two different strains were used together, equal amounts of each were added. The total amount of inoculum added to each pot was 75 gms. Twenty-five seeds of Red Bobs wheat were sown in each pot and covered with one inch of sterile soil. The plants were harvested and examined for perithecia 8 weeks after emergence. The results are presented in Table II.

The first part of the report is devoted to a description of the
 general situation in the country at the beginning of the year.
 It then goes on to discuss the various factors which have
 influenced the economic development of the country during the
 year.

Conclusion

In conclusion, it may be said that the year has been a year of
 progress and development for the country. The various factors
 mentioned above have all contributed to the growth and
 prosperity of the country. It is hoped that the same
 progress will continue in the future.

TABLE II

EFFECT OF MIXED INOCULUM ON PERITHECIAL PRODUCTION
AND PATHOGENICITY

Strains used as inoculum	Perithecial production	Pathogenicity rating
8	0	5
8; III-2	0	3
8; IV	0	3
8; S ₁	0	3
8; S ₂	0	3
Check (No inoculum)	0	0

Although infection was fair in all pots, no perithecia were observed and the pathogenicity of the mixed cultures was reduced over that of strain 8 alone.

Discussion

Colonies of mixed strains were moderately antagonistic in pure culture in these studies. This phenomenon was not exhibited by colonies of the same strain. Although this strain interaction exhibited was not characterized by

perithecial production as reported by Kirby, the appearance of a ruffled type of growth is interesting. It is unlikely that this phenomenon was due to a competition for nutrients since it was not observed to occur between colonies of the same strain. The interaction suggests some nutritive, hormonal or antibiotic activity between strains. Such an explanation might account for Kirby's conclusions that there are + and - strains of this organism.

In the pot studies, there was no interaction between different strains which stimulated perithecial formation on the host. The reduction of the pathogenicity of the mixed cultures over that of strain 8 alone might suggest some strain interaction. However these four strains are all less pathogenic than strain 8. Thus it is likely that the reduction of pathogenicity is due to a dilution effect similar to that observed by Henry and Gilpatrick (37) when working on the relative pathogenicity of single and mixed strains of this organism.

Studies with Different Strains

It seems likely that certain strains of this organism may be more fertile than others. Davis (16) observed that one of his strains would fruit readily in pure

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It is the policy of the Department of the Interior to make available to the public as much information as possible concerning the activities of the Department. This information is being furnished to you in accordance with the provisions of the Freedom of Information Act, 5 U.S.C. 552, and the Department's regulations thereunder, 43 CFR 161.001-1.

culture whereas two other strains were never observed to produce perithecia under the same conditions. Russell (72) and Davies (14) also observed that some strains produced perithecia more readily than others.

Many other fungi are composed of strains with different sporulating abilities. Leonian (51) in a study of Valsa leucostoma found this fungus to possess two strains: one capable of giving rise to both the perfect and imperfect stage; the other incapable of developing perithecia regardless of the environment. Different abilities of different strains to carry out asexual reproduction in pure culture have been observed by Hansen (28) for Botrytis, by Keitt and Langford (43) for Venturia inaequalis, and by Kernkamp (45) for Ustilago zaeae. Westergaard and Mitchell (83) developed a synthetic medium for perithecial formation by certain wild type crosses of Neurospora which was unsatisfactory for the fruiting of certain mutants with specific growth requirements.

The possibility also exists that O. graminis may lose its fertility in pure culture, particularly since this organism may lose its pathogenicity under the same conditions as has been observed by workers at this laboratory and by Russell (72) and since other fungi lose their fertility after a period in artificial culture.

Ryker (74) reported that cultures of certain species of Ceroospora ultimately produce feebly sporulating variants and that this was due to the suppression of the original cultures by non-conidial variants. Chilton (11) has reported a similar situation for Colletotrichum destructor. During an investigation of the physiology of perithecial production in Melanospora destruens, Hawker (32) found that the strain in use became progressively less fertile. This investigator observed that during subculturing at laboratory temperature growth of sterile saltants began earlier than that of fertile strains. However at higher temperatures this difference was greatly reduced.

Experimental Studies

The ability of different strains of O. graminis to sporulate was investigated as well as the possibility of the loss of fertility in artificial culture.

Over a period of two years, 18 different strains were never observed to sporulate on potato dextrose agar in pure culture. At times other media were also used. Cultures were always incubated at about 20°C in the greenhouse or near a north window in the laboratory. Ten of

1970-1971, showing that although the number of
underground libraries has increased, the number
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relatively low, and the number of libraries is
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the isolates had been cultured for almost 20 years. The other 8 cultures were freshly isolated.

In one experiment, 5 recent isolates were grown singly on agar media while strains S₁¹ served as a check. The two media used were wheat-stem agar and a basic nutrient medium² to which biotin and thiamin were added at the rate of 100 µg. and 1,000 µg. per litre respectively. The strains used and the relative number of perithecia-like black bodies produced are recorded in Table III.

TABLE III

RELATIVE ABILITY OF DIFFERENT STRAINS TO PRODUCE PERITHECIA-LIKE BLACK BODIES ON VITAMIN AND WHEAT STRAW AGARS

Strain ³	Vitamin agar	Wheat-stem agar
A45	None	Few
A47	Few	None
A49	None	None
A50	Few	None
A60	Many	Few
S ₁	Many	Few

1 = Isolated by S. Goto at this laboratory in 1943.

2 = Basic medium described on Page 82.

3 = The writer wishes to express his thanks to Dr. L. E. Tyner, Dominion Laboratory of Plant Pathology, Edmonton for kindly supplying the recent isolates used in these studies.

The tendency toward the production of the perithecia-like bodies on the media tested is apparently greatest with strain A60 and S₁. It is also interesting to note that these bodies were produced more abundantly on the vitamin agar than on the wheat-stem agar.

In another experiment, the relative ability of 6 isolates to sporulate on the host was investigated. Three strains - A49, A60, and A71 were recent isolates. The other strains had been in culture for 4 - 17 years.

Wheat plants were infected according to the flask method as described in the section on physical factors. In this method wheat seedlings are planted aseptically in small Erlenmeyer flasks containing the fungus growing on a mixture of soil and corn meal. In this experiment no attempt was made to maintain aseptic conditions after good infection was apparent and the bungs of the flasks were removed at that time exposing the cultures to contamination from the air.

After 8 weeks, the plants in the flask were examined for perithecia. The relative pathogenicity and perithecial production are presented in Table IV.

Under the conditions of this experiment, strains A71, A49, A60, and 8 were highly pathogenic, killing the host plant outright in most cases. However, strains III-17 and S₁

The following is a list of the names of the persons who have been appointed to the various committees of the Board of Directors of the Corporation.

Committee on Finance: Mr. J. H. Smith, Chairman; Mr. A. B. Jones, Secretary; Mr. C. D. Brown, Treasurer; Mr. E. F. Green, Member; Mr. G. H. White, Member.

Committee on Operations: Mr. I. J. Black, Chairman; Mr. K. L. Gray, Secretary; Mr. M. N. Blue, Treasurer; Mr. O. P. Red, Member; Mr. Q. R. Purple, Member.

Committee on Legal Affairs: Mr. S. T. Yellow, Chairman; Mr. U. V. Orange, Secretary; Mr. W. X. Green, Treasurer; Mr. Y. Z. Blue, Member; Mr. A. B. White, Member.

Committee on Public Relations: Mr. C. D. Brown, Chairman; Mr. E. F. Green, Secretary; Mr. G. H. White, Treasurer; Mr. I. J. Black, Member; Mr. K. L. Gray, Member.

Committee on Personnel: Mr. M. N. Blue, Chairman; Mr. O. P. Red, Secretary; Mr. Q. R. Purple, Treasurer; Mr. S. T. Yellow, Member; Mr. U. V. Orange, Member.

Committee on Audit: Mr. W. X. Green, Chairman; Mr. Y. Z. Blue, Secretary; Mr. A. B. White, Treasurer; Mr. C. D. Brown, Member; Mr. E. F. Green, Member.

Committee on Safety: Mr. G. H. White, Chairman; Mr. I. J. Black, Secretary; Mr. K. L. Gray, Treasurer; Mr. M. N. Blue, Member; Mr. O. P. Red, Member.

Committee on Environmental Affairs: Mr. Q. R. Purple, Chairman; Mr. S. T. Yellow, Secretary; Mr. U. V. Orange, Treasurer; Mr. W. X. Green, Member; Mr. Y. Z. Blue, Member.

Committee on Information Systems: Mr. A. B. White, Chairman; Mr. C. D. Brown, Secretary; Mr. E. F. Green, Treasurer; Mr. G. H. White, Member; Mr. I. J. Black, Member.

Committee on Compliance: Mr. K. L. Gray, Chairman; Mr. M. N. Blue, Secretary; Mr. O. P. Red, Treasurer; Mr. Q. R. Purple, Member; Mr. S. T. Yellow, Member.

were only slightly pathogenic which probably accounts for their inability to form perithecia. Strain S₁ has on the other occasions produced perithecia on the host when exhibiting moderate pathogenicity. Among the pathogenic strains, A60 was almost sterile, strain 8 moderately fertile, and strains A49 and A71 produced perithecia abundantly.

TABLE IV

RELATIVE PATHOGENICITY AND FERTILITY OF DIFFERENT STRAINS ON THE HOST

Strain	Replicates					
	a	b	c	d	e	f
A49	5 ++++	5 ++++	5 ++++	5 ++++	5 ++++	5 ++++
A60	5 -	5 +	5 -	5 -	5 -	5 -
A71	5 ++++	5 ++++	5 ++++	5 ++++	5 ++++	5 ++++
8*	5 +++	5 ++	3 -	5 ++	5 ++	
III-17	0 -	2 -	0 -	2 -	2 -	0 -
S ₁	1 -	2 -	2 -	2 -		

* = Strains 8 and III-17 isolated by F. R. Davies at this laboratory in 1931.

For meanings of figures and symbols see next page.

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TABLE

TABLE OF CONTENTS

PART I						PAGE
1	2	3	4	5	6	
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19	20	21	22	23	24	115
25	26	27	28	29	30	120
31	32	33	34	35	36	125
37	38	39	40	41	42	130
43	44	45	46	47	48	135
49	50	51	52	53	54	140
55	56	57	58	59	60	145
61	62	63	64	65	66	150
67	68	69	70	71	72	155
73	74	75	76	77	78	160
79	80	81	82	83	84	165
85	86	87	88	89	90	170
91	92	93	94	95	96	175
97	98	99	100	101	102	180

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Numerical figures represent pathogenicity rating.

- 0 = Non-pathogenic
- 1 = Trace of pathogenicity
- 2 = Slight pathogenicity
- 3 = Moderate pathogenicity
- 4 = Severe pathogenicity
- 5 = Plants killed

Algebraic signs represent relative perithecial production.

- = No perithecia
- + = Few spores observed when tissue was examined
- ++ = Few perithecia per plant (1 - 5)
- +++ = Several perithecia per plant (6 - 10)
- ++++ = Many perithecia per plant (more than 10)

Discussion

From the results of observations made on the relative ability of different strains to sporulate, it appears that some strains are more fertile than others under the same environmental conditions. These results also indicate that the culturing of strain 8 for 17 years has not caused this strain to become sterile. Furthermore, strain S₁, an isolate of many years, has produced perithecia

1. The first part of the report is devoted to a general survey of the situation in the country. It is followed by a detailed analysis of the economic situation, which shows that the country is in a state of economic crisis. The main reasons for this are the excessive expenditure on the military, the neglect of the economy, and the corruption of the government. The report concludes that the only way out of this crisis is a radical reform of the government and the economy.

2. The second part of the report is devoted to a detailed analysis of the economic situation. It shows that the country is in a state of economic crisis. The main reasons for this are the excessive expenditure on the military, the neglect of the economy, and the corruption of the government. The report concludes that the only way out of this crisis is a radical reform of the government and the economy.

Conclusion

The report concludes that the only way out of this crisis is a radical reform of the government and the economy. It is necessary to reduce the military expenditure, to neglect the economy, and to reform the government. The report also suggests that the country should be reformed into a democracy, and that the economy should be reformed into a free market economy.

on other occasions in these studies. Thus with these two strains at least, fertility has not been completely lost by their long culturing on an artificial medium.

The inability of strains A49, A71, and 8 to sporulate in pure culture is evidently not due to their infertility but more probably the result of improper nutritional or physical conditions. Favorable conditions are evidently provided by the host plant under the environment present in the experiment described.

The fact that single strains sporulated on the host in the present studies supports the observations of Davis (16), Davies (14), and White (84), that O. graminis is homothallic.

EFFECT OF PHYSICAL FACTORS ON SPORULATION OF FUNGI

Introduction

The physical elements of the environment such as light, humidity, and temperature seem to play their part in the reproductive life of the fungi. Many fungi sporulate well in nature where the physical conditions are evidently satisfied. However, in pure culture some fungi do not sporulate readily and it is possible that under these conditions some physical factor is not satisfactorily provided. The following literature review although incomplete illustrates the importance of light, temperature, and humidity on the sporulation of a few fungi.

Literature Review

Light

Probably the most extensively studied physical factor affecting the fruiting of fungi is light. Many workers have reported that light helps to initiate, increase,

MEMORANDUM

The first element of the program was
to study the history of the
university and to see how it
has changed over the years.
This was done by looking at
the records of the university
and by talking to the people
who have been involved in
its development. The result
was a book which tells the
story of the university from
its beginning to the present
day.

APPENDIX

1950

This appendix contains a list of
the names of the people who
have been involved in the
development of the university
from its beginning to the
present day.

and hasten sporulation. Ramsey and Bailey (65) found that a strain of Fusarium coeruleum which had never produced conidia in culture did so following exposure to ultra violet radiations. These same workers found that spore production of Macrosporium tomato and Fusarium cepae was increased greatly by exposure to the same radiations. Hutchinson and Ashton (39) obtained numerous acervuli and conidia of Colletotricum phomoides within 24 hours after radiating by means of a mercury arc lamp. Under normal conditions acervuli do not usually develop until the 8th day.

Light may modify spore characteristics. The conidia of three Fusarium species studied by Harter (29) reached their greatest length in cultures exposed to a maximum amount of daylight while those produced in the dark were shorter.

Dillon-Weston (19) demonstrated that the quantity of light may be of importance. With the several species studied he found that long exposures to ultra violet inhibited whereas short exposures stimulated sporulation. He also noted that under ordinary laboratory conditions the light intensity is not great enough to induce Helminthosporium avenae or Altenaria solani to sporulate. Snyder and Hansen (80) reported that Centrospora acerina sporulated profusely

The first part of the report deals with the general situation in the country. It is noted that the economy is in a state of stagnation and that the government has failed to implement the necessary reforms. The report also mentions that the population is suffering from poverty and unemployment.

The second part of the report discusses the political situation. It is noted that the government is corrupt and that there is a lack of transparency in its operations. The report also mentions that there is a growing opposition to the government and that there are calls for reform.

The third part of the report discusses the social situation. It is noted that there is a high level of inequality in the country and that the poor are being exploited by the rich. The report also mentions that there is a lack of access to education and healthcare for many people.

The fourth part of the report discusses the international situation. It is noted that the country is facing a trade deficit and that it is being isolated by the international community. The report also mentions that there are concerns about the country's stability and that there are calls for international intervention.

The fifth part of the report discusses the future of the country. It is noted that there is a need for comprehensive reforms and that the government must take action to address the country's problems. The report also mentions that there is a need for greater transparency and accountability in the government.

In conclusion, the report states that the country is in a state of crisis and that the government must take immediate action to address the country's problems. The report also mentions that there is a need for greater transparency and accountability in the government and that there is a need for comprehensive reforms.

on agar media outside a north window but not at all inside the window. The same is true of the imperfect stage of Botryosphaeria ribis and Mycosphaerella brassicola. Houston and Oswald (38) studied the influence of photoperiodism on the sporulation of Helminthosporium gramineum. They found that exposure to light - preferably daylight - is necessary for conidial development on agar.

Temperature

In a recent review of the literature on the effect of temperature on the reproduction of fungi, Wolf and Wolf (87) concluded that there is abundant evidence that temperatures favorable for the growth of fungi may be slightly lower than those for reproduction. They cite many illustrations of this relationship. However, Cherewick (10) found that chilling of plants infected with Erysiphe graminis increased perithecial formation and observed that alternating temperatures brought about greater sporulation.

Keitt and Langford (43) demonstrated that temperature greatly influences perithecial formation of Venturia inaequalis. Mature perithecia were produced abundantly in pure culture in about 5 months if incubated at 8°C.

The first part of the report deals with the general situation in the country and the progress of the work done during the year. It also contains a list of the names of the persons who have been appointed to various positions in the service of the Government.

Appendix

The following is a list of the names of the persons who have been appointed to various positions in the service of the Government during the year.

The names of the persons who have been appointed to various positions in the service of the Government during the year are as follows:

The names of the persons who have been appointed to various positions in the service of the Government during the year are as follows:

The names of the persons who have been appointed to various positions in the service of the Government during the year are as follows:

Fruiting was still abundant but took longer at 4°C. An abundance of initials but only an occasional mature perithecium with ascospores was found in cultures incubated at 12°C, while only small initials and no ascospores were produced at 16°, 20°, and 24°C, respectively. Further tests showed that mature perithecia could be obtained in a shorter time by incubating the plates at about 20°C for the first 10 days to produce rapid vegetative development then lowering the temperature to 8°C until the early ascus stage was reached and then raising the temperature to about 15°C. If however the temperature was raised before the perithecia were sufficiently advanced, no ascospores were formed.

Humidity

This factor seems to play an important part in the development and maturation of fruiting bodies. Sporangia of Phytophthora infestans form readily only in saturated atmospheres. No sporangia are formed by this organism at a relative humidity less than 91 percent (13). Alternate drying and wetting of perithecia is necessary to induce the formation of ascospores by Erysiphe graminis but other stages of the sexual process are favored by relatively dry conditions (10). Dixon et al. (20) observed that

sporulation of the tobacco downy mildew fungus (Peronospora hyoscyami) is favored by long periods of saturated humidity at a time when skies are overcast. Longree (55) demonstrated that decreasing the relative humidity gave sparser mycelial development and fewer conidiophores with Sphaerotheca pannosa var. rosae on young rose leaves, but that both events occurred even at 21 - 22% relative humidity.

Physical Factors and the Sporulation of O. graminis

Literature

There is little available information on the effect of physical factors on the sporulation of O. graminis. Davis (16) was unable to find perithecia in cultures of this organism incubated in the darkness but often observed them in cultures held in the daylight. However, he points out that occasionally perithecia are found on the roots of plants near the crown where they are formed in the darkness. Davies (14) reported that 4 strains which failed to sporulate in darkness, produced perithecia when transferred to the daylight of the laboratory. This author also studied the effect of ultra violet light but found that exposure from 2 to 15 seconds did not stimulate perithecial production to any great extent. Both Russell (72) and Garrett (25)

consider that light exerts a favorable influence on sporulation.

Russell (72) reported that exposures of cultures to low temperatures seemed to stimulate the production of perithecia. Davies (14) incubated cultures at different temperatures for 4 weeks and then transferred these to the laboratory where they were placed at room temperature in the light. No significant effect of temperature on fruiting was observed.

Moisture and humidity probably influence perithecial formation of O. graminis. As previously pointed out, perithecia are commonly found on diseased wheat plants in the British Isles and in the eastern United States where the relative humidity and rainfall are usually much greater than in Alberta where perithecia are less commonly observed. In New South Wales, Hynes (40) considers that the development of perithecia is dependent on adequate moisture supplies.

Experimental Studies

Methods of Inducing Sporulation

Techniques for inducing sporulation of O. graminis have been devised taking into consideration the effect of

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physical and other factors. A few of these methods have been investigated in these studies to ascertain their relative value.

Hynes' Method

In this method, Hynes (40) moistened the butts of wheat plants affected with Take-all collected from the field, and placed them in Erlenmeyer flasks under laboratory conditions. Fruitifications soon developed.

In testing this method, severely infected wheat seedlings were harvested from artificially inoculated soil in pots in the greenhouse. These plants were washed and placed in 250 cc. flasks with 10 seedlings per flask. A small amount of water was added to each container. Three flasks were then incubated under each of the following conditions:

- (1) In a humidity chamber in the greenhouse in diffuse sunlight at 15° - 20°C.
- (2) In the greenhouse in bright sunlight at 20° - 30°C.
- (3) In a north window in the laboratory at 18° - 22°C.
- (4) In a temperature control chamber in the laboratory at 18°C in the dark.

Moisture was added to these flasks as required.

At the end of 8 weeks these plants were examined but no perithecia were observed.

Garrett's Method

Perithecia were obtained in abundance by Garrett (23) when using the following method:

wheat seedlings were planted above agar inoculum of the fungus in large tubes containing sand moistened with a standard nutrient solution for wheat. The tubes were incubated in glass jars at 16° - 20°C in a north window. No attempt was made to maintain sterile conditions. Mature perithecia were obtained in about 6 weeks.

This method was tested on two occasions in the present studies, but unfortunately in both cases only poor infection resulted, so that the method could not be appraised.

White's Method

White (84) obtained favorable conditions for perithecial production by inoculating the roots of wheat plants in pure culture on nutrient agar in large tubes. Four weeks later the plants died and cultures were left to dry out. Mature perithecia developed in about 8 - 10 weeks and formed along the roots at the junction of the agar and glass.

To test this method, sterile wheat seedlings were added to potato dextrose agar slants upon which O. graminis was growing. The slants were incubated in diffuse light in the greenhouse. Although good infection resulted, no fruiting bodies appeared.

Possibly better success might have been achieved with this method if it had been tested further with the use of other media.

Flask Method

A successful method for producing perithecia on the host plant was devised during the present study. This consisted of growing wheat plants in small flasks containing artificially inoculated soil. Fifty grams of a mixture of soil and corn meal (10 percent by weight) was added to small Erlenmeyer flasks and moistened with 32 ccs. of distilled water. The flasks were bunged and sterilized in the autoclave at 15 pounds pressure for two hours. A small bit of O. graminis mycelium growing on an agar slant was used as inoculum. After a 21-day incubation period, the flasks were planted aseptically with 5 Red Bobs wheat seeds which had been previously surface sterilized with 1:1000 mercuric chloride and rinsed with sterile water. At this same time 5 ccs. of water was added to each container to provide adequate moisture for the growth of the host.

The first step in the process of the development of the national economy is the development of the agricultural sector. This is because agriculture is the basis of the economy and provides the raw materials for the other sectors. The government should therefore focus on improving the productivity of the agricultural sector through various measures such as providing credit facilities, technical assistance, and infrastructure development.

CONCLUSION

The development of the national economy is a complex process that requires the coordinated efforts of the government, the private sector, and the citizens. The government should play a leading role in creating a favorable environment for economic growth through sound policies and effective implementation. The private sector should be encouraged to invest in the economy and create jobs. The citizens should be educated and empowered to participate in the development process. Only through the concerted efforts of all these stakeholders can the national economy achieve sustainable growth and development.

These planted flasks were incubated in a humidity chamber in the greenhouse at from 15° - 20°C. Direct sunlight was never allowed to fall on the plants but light intensity during the day was high. Usually moisture was condensed on the inside of the flasks at least to a height of 20 cms. above the soil line.

Good infection was apparent 15 days after seeding. The plants were dead and perithecia could be observed at the end of 6 weeks.

Sterile conditions were maintained in about 50 percent of the flasks. A Helminthosporium species which was introduced through the seed was the most common contaminant. On some occasions the bungs were removed from the flasks after sowing the seed in order to allow recontamination of the soil. Infection was not appreciably reduced by recontamination when highly pathogenic strains were used.

Discussion

Of the four methods tested in these studies, the flask method was the only one which was used successfully for the production of perithecia. The success of this method was probably due in considerable measure to the fact that the substrate for the organism was the host plant, that good

The first thing I noticed when I stepped
 out of the car was the smell of
 fresh air and the sound of birds
 singing in the trees. It was a
 wonderful feeling, and I knew
 that I was in a good place.
 The house was just what I needed.
 It was quiet and peaceful, and
 I could see the mountains in the
 distance. I had found a new home.
 I had found a place where I could
 be myself and live in peace.
 I had found a place where I could
 be happy and content.
 I had found a place where I could
 be free and at last.
 I had found a place where I could
 be whole and complete.
 I had found a place where I could
 be at home.

Conclusion

In conclusion, the study has shown
 that the use of the proposed
 method is effective in solving
 the problem. The results are
 consistent with the theoretical
 predictions. The proposed method
 is simple and easy to use, and
 it can be applied to a wide range
 of problems. The study has
 provided a clear and concise
 explanation of the method and its
 application. The results are
 presented in a clear and concise
 manner, and the conclusions are
 well supported by the data.

infection was obtained, that the relative humidity was high, and that abundant daylight was provided. Garrett (23) concluded that these same conditions contributed to the success of his method. He also considered that the maintenance of non-sterile conditions was important. Possibly the non-sterile conditions often prevailing when the flask method is used contribute to its success. This point will be more fully discussed in the section dealing with the microorganismal factor.

Each of the methods described involved the use of the living host plant as the substrate. Such methods have definite limitations. A general lack of uniformity exists resulting from biological variations among different plants. It is difficult to establish uniform infection in each plant. Furthermore, any environmental factors acting on the organism are also acting on the substrate or host plant. Thus the substrate is not likely to be uniform under different conditions of environment. A method making use of a substrate that could be duplicated would be preferable.

The Effect of Light and Diurnal Effects on Sporulation

An attempt was made to determine the importance of outdoor environment on the sporulation of O. graminis.

Ten slants each of 3 percent malt agar and potato dextrose agar were inoculated with strain S₁ and placed outdoors in contact with the soil and exposed to daylight. Ten inoculated slants of each medium were also retained in the laboratory at room temperature. Rather cool conditions, 5° - 25°C, prevailed outdoors during the experiment.

No perithecia were observed after 60 days in either the outdoor or indoor cultures. Very rough growth occurred on malt agar under both conditions and numerous black, perithecia-like bodies were formed.

The outdoor cultures were left out for another 4 weeks during which time freezing conditions occurred on a few nights. These cultures were then returned to the laboratory and incubated at room temperature in a north window. After two weeks they had dried out. At this time they were revived by adding water and at the end of 10 days were examined for perithecia but none were found.

Evidently the conditions of the laboratory or the field did not provide a satisfactory environment for perithecial formation by this strain on the media tested.

The effect of photoperiodism on sporulation was also studied. Wheat plants were infected with the organism using the flask method and maintaining as sterile

conditions as possible. After the plants had become severely infected and were starting to die, six flasks each containing 5 plants were placed under each of the following light environments with temperature and humidity held constant:

- (1) continuous darkness
- (2) 4-hour day
- (3) 8-hour day
- (4) 12-hour day

At the end of 4 weeks, the plants were examined for perithecia and the lengths of ascospores under each light environment were measured. The results are presented in Table V.

TABLE V

THE EFFECT OF LIGHT DAYS OF DIFFERENT LENGTH
ON RELATIVE PERITHECIAL AND ASCOSPORE PRODUCTION AND
ASCOSPORE LENGTH

Treatment	Number perithecia	Number ascospores	Ascospore length in microns	
			Range	Mean length (100 spores)
Continuous darkness	++++	+	56-79	67.9 ± 3.4
4-hour day	++++	++++	60-80	68.5 ± 3.1
8-hour day	++++	++++	65-78	64.5 ± 3.7
2-hour day	++++	++++	62-80	70.5 ± 3.6

- The first part of the report deals with the general situation of the country and the progress of the work done during the year. It also mentions the various committees and their work.

- (i) General
- (ii) Finance
- (iii) Education
- (iv) Health
- (v) Agriculture
- (vi) Industries
- (vii) Social Services
- (viii) Miscellaneous

- The second part of the report deals with the various committees and their work. It mentions the names of the members and the work done by each committee.

ANNEXURE

- The following are the names of the members of the various committees mentioned in the report.

Committee	Members	Chairman	Secretary	Members	Members
General	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100				
Finance	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100				
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Miscellaneous	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100				

Length of day had little effect on the relative number of perithecia or ascospores produced. Furthermore there were as many perithecia produced in darkness as in the light. However few perithecia formed in the dark bore mature ascospores. The length of day had no marked effect on ascospore length. However it is interesting to note that the mean spore lengths under these conditions were much shorter than for those formed in a normal day (See page 5).

Light evidently stimulates the maturation of perithecia and the production of ascospores by this fungus. In this experiment, possibly perithecia had started to form at the time the plants were placed in the various light environments or possibly the host or fungus had received some stimulus which induced perithecial formation later. Further exposure to light was probably necessary before these perithecia could produce an abundant supply of ascospores.

Light had a marked effect on the length and direction of growth of the beaks of the perithecia. In the dark, beaks were shorter and undeveloped; those in the light were long and slender and often bent upward toward the light. This phototropic effect is illustrated in Figure 3.

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The Effect of Other Physical Factors on Sporulation

Temperature

Although a few experiments were conducted on the effect of temperature on sporulation, results were always unsatisfactory and not worthy of recording here. However, it was generally observed that perithecia were formed at relatively cool temperatures (15°C). This agrees with Russell's suggestion (72) that sporulation of O. graminis is favored by cool conditions.

The optimum growing temperature for strain 8 ranged from 15° - 25°C . Fair growth occurred at 10°C and very poor growth at 30° and 35°C .

Humidity

Though^{no} special humidity experiments were made, it was noted that perithecia were formed only when the humidity was high and when the host or soil was not allowed to dry out.

Soil Moisture

An experiment was conducted to test the effect of soil moisture on sporulation. Wheat seedlings were infected by sowing seed in pots containing soil artificially inoculated with 5 different strains of O. graminis. Early in July, after infection had occurred, the seedlings were planted outside in natural soil in two lots and fully exposed to the elements. One lot was watered heavily every 2 or 3 days whereas the other was left unwatered. For the first 3 weeks in the field, conditions were very dry and there was a great difference in the soil moisture content of the watered and unwatered plots. However a wet season followed and continued into September. At the end of this month all plants were harvested and examined for perithecia but none were found even though many plants were severely lesioned.

It is interesting to note that perithecia did not form on any of the diseased plants although in the case of one lot, the moisture content of the soil was high throughout the entire period.

1941-1942

The following is a summary of the work done during the year 1941-1942. The work was done in the laboratory of the Department of Zoology, University of Toronto, Ontario, Canada. The work was done under the supervision of Dr. J. H. Spongberg. The work was done during the months of August, September, October, November, and December, 1941, and January, February, March, April, May, and June, 1942. The work was done in the laboratory of the Department of Zoology, University of Toronto, Ontario, Canada. The work was done under the supervision of Dr. J. H. Spongberg. The work was done during the months of August, September, October, November, and December, 1941, and January, February, March, April, May, and June, 1942.

Discussion

From the results of these experiments and from the reports in the literature it appears that physical factors play a large part in the sporulation of O. graminis. The role of light appears to be quite significant. Light was shown to be necessary for the maturation of perithecia and to have a marked effect on the morphological characters of the perithecia.

In the experiments reported, perithecia were only produced on the host when abundant light, high humidity, and fairly cool temperatures were provided. When studying other factors, these physical conditions were maintained as closely as possible to these levels in an attempt to supply favorable physical conditions for sporulation.

The failure of 5 strains of the O. graminis to sporulate on plants growing in soil with a high moisture content suggests that this factor is not the only one which governs sporulation of this fungus.

The first part of the report is devoted to a general
 description of the country and its resources. It
 is followed by a detailed account of the
 various industries and occupations of the
 people. The third part of the report
 contains a list of the principal towns and
 villages of the country. The fourth part
 of the report contains a list of the
 principal rivers and streams of the
 country. The fifth part of the report
 contains a list of the principal mountains
 and hills of the country. The sixth part
 of the report contains a list of the
 principal lakes and ponds of the
 country. The seventh part of the report
 contains a list of the principal forests
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 of the report contains a list of the
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 tenth part of the report contains a list
 of the principal plants of the country.

THE EFFECT OF MICROORGANISMAL FACTORS ON SPORULATION

Literature Review

It is a well established fact that many fungi sporulate more readily or more profusely when in association with other microorganisms in artificial culture than when grown alone. Asthana and Hawker (2) have reviewed the literature dealing with this subject up until 1936. They also report that sporulation of Melanospora destruens and some other Ascomycetes was stimulated by the presence in the culture plates of certain organisms or products derived from the latter. Gwynne-Vaughan (27) in a review of the question of sex and nutrition of the fungi concluded that heterothallism in Humaria granulata apparently is not based on a difference in sex but on a difference in metabolism of the one strain brought about by the presence of the second. Nickerson and Thimann (59) showed that Aspergillus niger produces a substance which promotes conjugation of three Zygosaccharomyces spp. which conjugate only sparingly

in pure culture. Dead cells of these Zygosaccharomyces contain some of this substance which probably induces some conjugation.

Hazen (36) has recently demonstrated that Microsporium audouini is deficient in certain factors essential for its profuse growth and for the development of macroconidia. These factors may be produced by a bacterium, Bacillus weidmaniensis.

Sporulation of O. graminis is also influenced by other organisms with which it associates. In his Master's thesis presented to the University of Alberta in 1932, Davies (14) reported that the presence of an unidentified bacterium stimulated the abundant production of perithecia by O. graminis in artificial culture. He found that only the living bacterium had this stimulatory effect. Sterile extracts from liquid media in which the bacteria had grown did not induce perithecial formation. The foregoing and other findings on this subject made at this laboratory have been reported recently by Davies et al. (15). In one experiment 96 percent of wheat seedlings grown in unsterilized artificially inoculated soil produced perithecia, whereas only 8 percent of the seedlings in sterilized soil produced them. These same workers concluded that "the

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ability to stimulate sporulation does not appear to be possessed by many individual microorganisms since a survey of more than 450 isolates of fungi, bacteria, and actinomycetes from the soil, the basal parts of wheat plants, and other sources failed to disclose this property in any of them".

Reviews of the extensive literature dealing with the effect of other microorganisms on growth, pathogenicity, and survival of O. graminis are presented by Garrett (25) and Slagg and Fellows (78).

Broadfoot (7) studied the growth relationships of 66 cultures of bacteria and fungi toward O. graminis on agar media and on the host. He found that any growth reaction on the two solid media used was not a reliable indication of what happens on the host and that different growth relationships occur on different media. The suggestion was made that various microorganisms, each with its complement of enzymes, might produce substances which differ in kind and amount according to the substrate.

Slagg and Fellows (78) found that some fungi produce substances that inhibit the growth of O. graminis in pure culture, whereas the byproducts of other fungi act in a stimulatory manner. They observed that the

ability to establish a position does not seem to be

indicated by any analysis of the data.

There is some evidence that the ability to

establish a position is related to the ability to

establish a position. This is shown by the

results of the analysis.

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production of these products by any fungus varied with its stage of growth and with the nature of the substrate. They also noted that some fungi may produce inhibitory byproducts on one culture medium and stimulatory byproducts on another. Neither these workers nor Broadfoot reported on the effect of other microorganisms on the sporulation of O. graminis.

Experimental Studies

The following experiments were conducted in an attempt to obtain further information on the effect of various microorganisms on the growth and sporulation of O. graminis.

Experiment I

The effect of 98 different isolates on the sporulation of O. graminis was studied on potato dextrose agar in Petri plates. Seventy-three of these organisms were isolated from the soil around wheat stubble gathered

The first of these is the fact that the
 Government has not yet decided whether
 to issue a writ of habeas corpus in
 this case. It is clear that the
 Government has a duty to act in
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 the duty of the courts to ensure that
 the Government does so.

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Footnote 1

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 it is the duty of the courts to
 ensure that the Government does so.

from the field. The isolates included 11 fungi, 24 actinomycetes, and 38 bacteria. The remaining 25 organisms used were available in the laboratory as named isolates and included the following:

Bacteria

<u>Bacillus megatherium</u>				<u>Aerobacter aerogenes</u>
<u>B. mycoides</u>				<u>Pseudomonas atrofaciens</u>
<u>B. mesentericus</u>				<u>Pseudomonas coronafaciens</u>
<u>B. niger</u>				<u>Erwinia amylovora</u>
<u>B. brevis</u>				<u>Erwinia carotovora</u>
<u>B. cereus</u>				<u>Phytomonas insidiosa</u>
<u>B. vulgatus</u>				<u>Phytomonas tumefaciens</u>
<u>B. subtilis</u>	-	strain	A16	<u>Corynebacterium sepedonicum</u>
<u>B.</u>	"	-	" A30	<u>Fungi</u>
<u>B.</u>	"	-	" A31	<u>Sclerotinia</u> sp.
<u>B.</u>	"	-	" A32	<u>Penicillium</u> sp.
<u>B.</u>	"	-	" A33	<u>Cladosporium</u> sp.
<u>B.</u>	"	-	" A231	

The inoculum of the above organisms was introduced onto the agar medium about 30 mms. distant from a week old colony of O. graminis (strain 8). The plates were incubated in the

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greenhouse in a humidity chamber. They were examined at the end of 8 weeks. No perithecia were observed.

The inability of the 98 organisms tested to induce O. graminis to sporulate in agar culture seems to support the suggestion of Davies et al (15) that very few organisms have this ability.

Experiment 2

1. Plate Studies

A study was made of the effect of time of inoculation on the interaction between O. graminis and other organisms in agar culture. Petri plates containing 25 ccs. of potato dextrose agar were planted with a bit of culture of strain 8. One series of these plates was also planted with 8 other organisms (one per plate). All plantings were about 30 mms. apart. A second series was planted when the O. graminis cultures were 5 days old; a third at 10 days. Trichoderma was added at the end of 5, 10, and 15 days. There were 5 replicates of each pair of cultures at each date.

The organisms used included the following:

O. graminis (strain S₁); a highly pathogenic strain of Helminthosporium sativum and ^{one} of a Fusarium species both

The undersigned, being duly sworn, deposes and says that he is the
 author of the within and foregoing report, and that the same is a true and
 correct copy of the original thereof, as the same appears by the
 original thereof, which is in his possession and control, and that
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 possession and control.

Subscribed and sworn to before me this _____ day of _____ 19____.

NOTARY PUBLIC

To all whom these presents shall come, I, the undersigned, do hereby
 certify that the within and foregoing report is a true and correct
 copy of the original thereof, as the same appears by the original
 thereof, which is in my possession and control, and that the same
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Witness my hand and seal this _____ day of _____ 19____.

My commission expires this _____ day of _____ 19____.

isolated from the basal parts of wheat plants; a yeast; two bacteria - 012 and 015 - from wheat stubble; a bacterium - 05 - known to stimulate the growth of O. graminis in a biotin free medium; a bacterium - ORS - capable of increasing the sporulation of a fungus isolated from a lesion on an orange fruit; and a Trichoderma species from the soil.

After inoculation with O. graminis all plates were incubated in the light of the laboratory for 3 weeks. They were then placed in the greenhouse in the diffuse light of a humidity chamber held at 15° - 20°C.

Observations on growth interaction were made at the end of 3 weeks and on perithecial formation after 6 weeks. The results are presented in Table VI and are illustrated in Figures 5 - 9.

The method of describing the growth interaction is the same as that used by Broadfoot (7). Any two colonies are considered as antagonistic or compatible. These two divisions are subdivided in the following manner:

I. Antagonistic

- A. A distinct neutral zone between O. graminis and the introduced organism.
- B. A distinct line of demarcation between O. graminis and the introduced organism.

The following information was obtained from the records of the Department of the Interior, Bureau of Land Management, and the Bureau of Reclamation, regarding the land acquisition project in the State of California.

The project involves the acquisition of certain parcels of land located in the County of [County Name], State of California. The parcels are situated within the [Area Name] area, and are being acquired for the purpose of [Purpose]. The acquisition is being carried out under the authority of the [Authority].

The parcels to be acquired are described as follows:

1. [Parcel Description]
2. [Parcel Description]

II. Compatible

- A. O. graminis grows over the introduced organism.
- B. Introduced organism grows over O. graminis.

The table and illustrations show that the Fusarium sp., the yeast, and the bacterium ORS were compatible with strain 8 at the three dates of testing. H. sativum and strain S₁ were antagonistic and Trichoderma completely inhibitive at all three dates.

There was a great difference in the growth reaction between plantings made at different times. This is well illustrated by bacterium OS which overgrew O. graminis at 0 days but caused inhibition when planted later. Bacterium O15 overgrew O. graminis at 0 days but was inhibited if planted after that.

In spite of the great variation of growth interaction of the various microorganisms at the different dates there were no perithecia produced by O. graminis. This provides further support for the suggestion of Davies et al (15) that ability to stimulate sporulation of O. graminis appears to be a property possessed by relatively few individual microorganisms. These differences emphasize the importance of age of culture in relation to the production of stimulatory and antibiotic substances, and the resulting

interaction. These results agree in general with those of Slagg and Fellows (78) and Broadfoot (7) who concluded that the interaction of O. graminis and another microorganism under one set of conditions is no criterion of what it may be under other conditions.

Possibly in plate studies similar to those reported here, more organisms could be found which would induce O. graminis to sporulate if the time of the addition of the inoculum of the introduced organism or other conditions were varied.

2. Flask Studies

Red Bobs wheat seedlings were infected according to the flask method previously described using as aseptic methods as possible. Five days after seeding, the same microorganisms used in the previous plate studies were added singly to the flasks by placing a bit of agar culture at the base of each seedling. In one treatment a bit of natural unsterilized soil was reintroduced. There were 12 replications of each culture treatment and 20 of O. graminis alone. Some flasks were discarded because contamination inhibited infection completely. Eight weeks after seeding, the plants were examined for perithecia. At the same time a bit of soil from each flask was streaked

Investment in the United States is expected to be high in 1954 and 1955, and to continue to rise in 1956 and 1957. This is due to the fact that the United States is expected to be a major source of capital for the rest of the world. The United States is expected to be a major source of capital for the rest of the world. The United States is expected to be a major source of capital for the rest of the world.

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TABLE VI

THE EFFECT OF TIME OF INOCULATION OF VARIOUS CULTURES ON THEIR INTERACTION WITH OPHIOBOLUS GRAMINIS SACC. ON POTATO DEXTROSE AGAR, AND THE EFFECT OF THESE CULTURES ON THE SPORULATION OF O. GRAMINIS IN PURE CULTURE AND ON WHEAT GROWING IN FLASKS

Added Cultures	Interaction on agar plates			Perithecial production in flasks							
	No. days plates inoculated after <u>Ophiobolus</u>			No. perithecia	No. flasks	No. flasks with perithecia	% flasks with perithecia	No. flasks contaminated	No. contaminated, with perithecia	No. not contaminated, with perithecia	Mean pathogenicity rating
	0	5	10								
Check-strain-8* (Fig. 5)	11-A	11-A	11-A	0	19	8	42	4	0	8	4.0
<u>O. graminis</u> -S ₁ (Fig. 5)	1-B	1-A	1-A	0	11	5	45.5	4	3	2	4.6
<u>H. sativum</u> -6 (Fig. 6)	1-A	1-B	1-A	0	9	4	44.5	3	1	3	4.1
<u>Fusarium</u> sp.-6 (Fig. 6)	11-B	11-A	11-B	0	11	1	9	8	1	0	4.0
Yeast (Fig. 7)	11-B	11-A	11-A	0	9	3	33.5	5	2	1	4.3
Bacterium-012 (Fig. 7)	11-A	1-A	1-A	0	9	7	77.5	5	2	5	4.1
Bacterium-0S (Fig. 8)	11-B	1-A	11-A	0	10	3	30	0	0	3	4.3
Bacterium-015 (Fig. 8)	11-B	1-A	1-B	0	9	2	22	2	0	2	4.1
Bacterium-ORS (Fig. 9)	11-A	11-A	11-A	0	10	4	40	5	2	2	4.0
<u>Trichoderma</u> sp. (Fig. 9)	--	z	z	0	10	0	0	0	0	0	0
Contaminated with natural soil	--	--	--	--	11	1	9	9	--	--	3.1

Pathogenicity rating -

0 = non pathogenic
5 = plants killed
1-4 = intermediate stages

*Checks were inoculated with O. graminis-8

z = Ophiobolus inhibited completely

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Figure 5

Association effects of different colonies of Ophiobolus graminis - effect of time of planting of introduced organism on the growth reaction of strain 8 on potato dextrose agar. Plantings left to right at 0, 5, and 10 days.

Upper row - O. graminis - 8 vs. O. graminis - 8.
Note compatibility of colonies.

Lower row - O. graminis - 8 (on left) vs. O. graminis S₁ (on right). Note the slight antagonism between colonies and the rough type of growth exhibited by strain S₁.

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Figure 6

Association effects of other organisms on Ophiobolus graminis - effect of time of planting of introduced organism on the growth reaction of Ophiobolus graminis - 8 on potato dextrose agar. Plantings left to right at 0, 5, and 10 days.

Upper row - O. graminis - 8 (on right) vs. Helminthosporium sativum - 6 (on left).

Lower row - O. graminis - 8 vs. Fusarium sp. - 6.



Figure 7

Association effects of other organisms on Ophiobolus graminis - effect of time of planting of introduced organism on the growth reaction of Ophiobolus graminis - 8 on potato dextrose agar. Plantings left to right at 0, 5, and 10 days.

Upper row - O. graminis - 8 (on right) vs. yeast (on left).

Lower row - O. graminis-8 (on right) vs. bacterium - 012 (on left). Note compatibility at 0 days, antagonism at 5 and 10.

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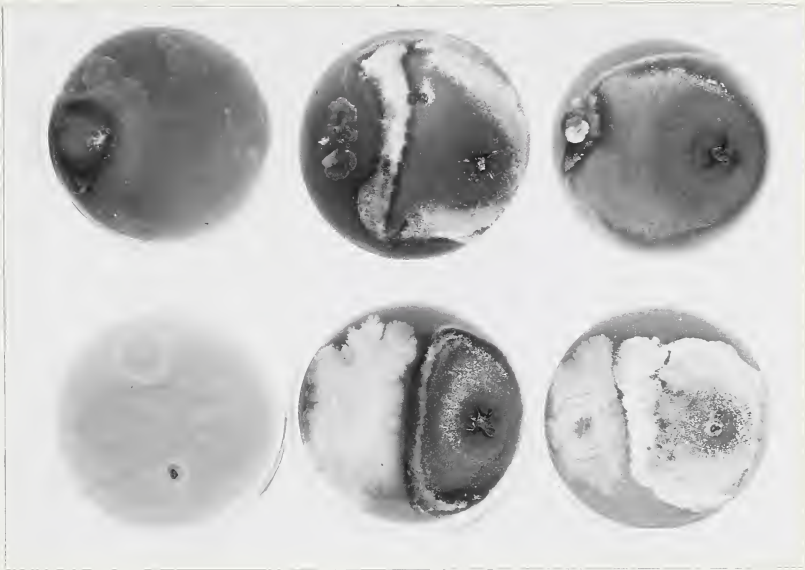


Figure 8

Association effects of other organisms on Ophiobolus graminis - effect of time of planting of introduced organism on the growth reaction of Ophiobolus graminis - 8 on potato dextrose agar. Plantings left to right at 0, 5, and 10 days.

Upper row - O. graminis - 8 vs. bacterium - 0S.

Lower row - O. graminis - 8 vs. bacterium - 015.

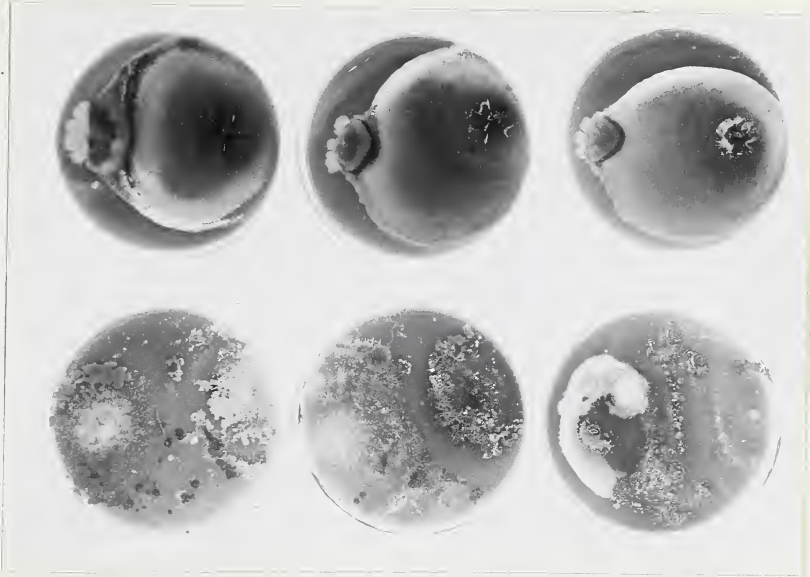


Figure 9

Association effects of other organisms on Ophiobolus graminis - effect of time of planting of introduced organism on the growth reaction of Ophiobolus graminis - 8 on potato dextrose agar.

Upper row - O. graminis - 8 vs. bacterium - ORS.
Plantings of bacterium, left to right,
at 0, 5, and 10 days.

Lower row - O. graminis - 8 vs. Trichoderma sp.
Plantings of Trichoderma, left to right,
at 5, 10, and 15 days. Note that O.
graminis is overgrown at first two dates
of planting.

on an agar slant to determine the sterility of the contents. Usually the introduced organism was reisolated. A summary of the observations made is presented in Table VI.

Of the 9 organisms tested only the bacterium 012 appeared to stimulate greater perithecial production by O. graminis. The *Fusarium* sp. seemed to have an inhibitory effect. The contents of unsterile soil apparently reduced the pathogenicity of O. graminis and perithecial production. Trichoderma overgrew the flasks and inhibited O. graminis completely. Seven of the remaining 8 organisms had little effect on the pathogenicity of O. graminis-8, but another strain of O. graminis (S₁) seemed to stimulate it.

The results of this experiment do not agree with the suggestion of Davies et al (15) that unsterile conditions tend to favor sporulation. There were fewer perithecia in flasks contaminated either purposely or accidentally with a composite microflora than in sterile checks. The importance of individual organisms is emphasized since one bacterium, 012, seemed to increase perithecial formation. This result is comparable to that of Davies (14) who found a bacterium capable of inducing sporulation in pure culture. Davies' bacterium and 012 could not be compared as the former was not available at the time these studies were conducted.

Experiment 3

Possibly the microflora associated with the wheat plant in the field may induce sporulation. Furthermore, perithecia are often found late in the season on infected dead wheat stubble and it is possible that some nutrient or the microflora present at that time may have a particular stimulatory effect.

In order to investigate these possibilities, wheat stubble was gathered from a field in the fall and planted in association with diseased wheat seedlings in small cardboard boxes in the greenhouse. The diseased seedlings were harvested from artificially inoculated soil and washed free of soil. Some were then wrapped around the unwashed stubble before planting in the boxes containing sterile soil, while others were planted alone. The boxes were placed in a humidity chamber in the greenhouse. The soil was kept saturated with water. All plants died in about 3 weeks time. At the end of 8 weeks, the plants were harvested and examined for fruiting bodies.

Perithecia were not observed on any of the plants. Abundant sporulation of Wojnowicia graminis occurred on the wheat stubble.

Evidently under the conditions of this experiment, the wheat stubble or its microflora could supply no stimulatory substance in satisfactory quantities to induce O. graminis to sporulate.

Experiment 4

A further attempt was made to determine the effect of the natural microflora of the soil on sporulation. Twenty-five wheat seeds were planted in pots containing sterile soil artificially inoculated with strain 8. When good infection was apparent, one set of 8 pots was recontaminated by leaching the soil twice with a suspension of natural soil in water. Another set was leached with sterile water.

About 8 weeks after seeding the plants were harvested and examined. At the same time the relative abundance of the microflora of the sterile and recontaminated pots was determined by the dilution plate method.

No perithecia were observed on any of the plants. Approximately the same number of colonies of bacteria and fungi were isolated from the soil in each set of pots but there was a much greater variety of organisms, from the recontaminated soil than from the 'sterile' soil.

1. The first part of the report is devoted to a general survey of the situation in the country, and to a description of the main features of the economy.

2. The second part

is devoted to a detailed analysis of the various branches of the economy, and to a study of the factors which have influenced their development.

The third part of the report is devoted to a study of the social and cultural conditions of the country, and to a description of the main features of the social structure.

This experiment suggests that the mere provision of unsterile conditions in the soil does not necessarily supply the mechanism for perithecial production of O. graminis.

Throughout this experiment the relative humidity was not held at a high level and this might explain the lack of sporulation. On the other hand the soil was kept well watered at all times and the plants never dried out.

Discussion

The results of the experiment reported here suggest that unsterile conditions are not necessary for sporulation of O. graminis on the host. Perithecia were formed under sterile or relatively sterile conditions. Recontamination of sterile soil or the association of diseased seedlings with unsteriled wheat stubble had no apparent stimulatory effect on fruiting. However, a few individual organisms may be stimulatory - a bacterium was found which seemed to increase fruiting on the host.

Davies (14) found a bacterium which induced perithecial production in pure culture. Possibly in nature perithecial development depends (other conditions being favorable) on the presence of certain individual organisms in sufficient quantities in association with diseased plants.

Any factor such as soil type, pH, and moisture content, which would influence the relative number of these organisms present in the soil or the production of their stimulatory substances would also likely influence perithecial production by O. graminis. This possibility might explain why perithecia are not always found in nature on plants infected with the Take-all fungus. This also might account for the discrepancy of observations made by Davies et al (15) and those made in these studies on the effect of unsterile conditions in the soil on sporulation.

THE EFFECT OF NUTRITION ON SPORULATION

Introduction

The nutritional conditions necessary for reproduction of fungi are not well known. However, the growth requirements are better understood and have been discussed recently in a review by Wolf and Wolf (87). Some of the elements often required by fungi for growth and incorporated into media as inorganic salts include sodium, potassium, phosphorus, magnesium, sulfur, iron, copper, zinc, and boron. The nitrogen requirements may often be satisfied by the inorganic form; at times the organic form is necessary. Usually carbon is supplied as a hexose sugar or as a disaccharide. With some fungi other substances may be required before growth occurs. These may include the vitamins or other growth factors.

Most fungi including *O. graminis* must produce vegetative growth or mycelia before reproductive bodies may arise. Thus, possibly, any nutritional factor affecting growth will also affect reproduction. However, this

THE HISTORY OF THE UNITED STATES

CHAPTER I

The history of the United States is a story of a people who have grown from a small group of immigrants to a great nation. The first settlers came to the eastern coast of North America in the early 17th century. They were seeking a new life and a better future for themselves and their families. Over the years, the number of settlers increased, and they began to establish permanent communities. These communities were often small and isolated, but they were the seeds of a new nation.

The early years of the United States were marked by a period of exploration and discovery. Explorers like Christopher Columbus and John Cabot had already discovered the Americas, but it was the English who first established a permanent settlement in North America. In 1607, the first English colony was founded in Jamestown, Virginia. This colony was the first of many that would follow, each bringing with it a different culture and way of life.

As the colonies grew, they began to develop a sense of identity and independence. They were no longer content to be ruled by a distant king in England. They wanted to govern themselves and make their own laws. This led to a period of conflict with England, which culminated in the American Revolution. The revolution was a struggle for freedom and self-determination, and it resulted in the birth of a new nation.

The new nation was born in 1776, and it has since grown into a great power. It has expanded its territory across the continent, and it has become a leader in the world. The United States has a rich history and a bright future. It is a nation of freedom, justice, and opportunity for all.

direct association has seldom been proved and there is evidence to show that this supposition does not necessarily apply (3).

The following account includes a fairly extensive review of the literature dealing with the effect of nutrition on the sporulation of the fungi as a whole. This is followed by a description of several studies made at this laboratory on the relationship between nutrition and the development of O. graminis. Although experiments were designed primarily to induce sporulation of the organism, if possible, observations were made on the effect of various nutritional factors on growth and development of perithecia-like black bodies.

Literature Review

Inorganic nutrients

Available information on the effect of the individual elements on sporulation is extremely limited. Asthana and Hawker (2) reported that phosphorous is essential for good growth and perithecial production of

The first part of the report deals with the general situation in the country and the progress of the work of the Commission. It is followed by a detailed account of the work done during the year, and a summary of the results achieved. The report concludes with a list of recommendations for the future.

The Commission has during the year been engaged in a number of important tasks, and has made considerable progress in its work. It has held several meetings, and has received many suggestions from the public. It has also carried out a number of investigations, and has published several reports.

The results of the work done during the year are as follows:

1. The Commission has held 12 meetings, and has received 150 suggestions from the public.

2. It has carried out 10 investigations, and has published 5 reports.

3. It has received 100,000 kroner in contributions from the public.

4. It has published 100,000 copies of its reports.

5. It has received 100,000 copies of its reports.

The Commission has during the year been engaged in a number of important tasks, and has made considerable progress in its work. It has held several meetings, and has received many suggestions from the public. It has also carried out a number of investigations, and has published several reports.

Summary of the work done during the year

Recommendations for the future

The Commission recommends that the Government should...

The Commission recommends that the Government should...

The Commission recommends that the Government should...

The Commission recommends that the Government should...

Melanospora destruens, Gilbert and Hickey (26) promoted sporulation of Penicillium notatum in submerged culture by adding iron in small quantities to the solution. Recently, Shu and Johnson (77) have shown that high concentrations of Zn , NH_4NO_3 , and KH_2PO_4 retarded spore formation of Aspergillus niger, but increasing the concentration of Mn favored sporulation.

Nitrogen Source

According to Coons (12), widely different classes of compounds may serve as the nitrogen source for pycnidium formation by Plenodomus fuscomaculans.

Leaver and his associates (50) after studying the nutritional requirements of Piricularia oryzae concluded that a large number of diverse nitrogenous compounds are effective in supporting conidial formation but that the amino-acids are required for full activity. This amino-acid requirement is apparently satisfied by glycine alone.

Of several nitrogen sources tested, Westergaard and Mitchell (83) found KNO_3 most satisfactory for the sporulation of Neurospora.

The first part of the report deals with the general situation of the country and the progress of the work done during the year. It then goes on to discuss the various departments and the work done in each of them. The report concludes with a summary of the work done and a list of the recommendations made.

RECOMMENDATIONS

It is recommended that the following steps should be taken to improve the efficiency of the work done during the year:

1. To improve the organization of the work done during the year.
2. To improve the methods of work done during the year.
3. To improve the personnel of the work done during the year.
4. To improve the equipment of the work done during the year.
5. To improve the materials of the work done during the year.
6. To improve the facilities of the work done during the year.
7. To improve the supervision of the work done during the year.
8. To improve the control of the work done during the year.
9. To improve the accounting of the work done during the year.
10. To improve the reporting of the work done during the year.

It is also recommended that the following steps should be taken to improve the efficiency of the work done during the year:

1. To improve the organization of the work done during the year.
2. To improve the methods of work done during the year.
3. To improve the personnel of the work done during the year.
4. To improve the equipment of the work done during the year.
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9. To improve the accounting of the work done during the year.
10. To improve the reporting of the work done during the year.

Leonian and Lilly (52) reported that aspartic acid is a satisfactory nutrient for zygospore formation in Phycomyces blakesleeanus. Glycine, arginine, α -alanine, and asparagine by themselves are poor sources of nitrogen but under the influence of succinic acid they induce a large number of zygospores. Ammonium nitrate, however, while greatly stimulating growth completely suppressed spore formation under certain conditions even when all necessary nutrients were present. Leonian (51) found that certain nitrates also reduced pycnidial formation and prevented perithecial production of Valsa leucostoma.

Carbon Source

Hawker and co-workers have presented a series of papers on the sporulation of certain Ascomycetes in relation to their carbon source. Seven of these fungi were essentially similar in their reaction to glucose and fructose. Growth and fruiting increased with an increase in hexose sugars up to a concentration, varying with species, above which fruiting fell off while mycelial growth continued to increase. The response to more complex carbohydrates showed more variation and was of three types: one similar to that

The following is a list of the names of the persons who have been appointed to the various committees of the Board of Directors of the National Bank of Commerce, New York, for the year ending December 31, 1910.

The Board of Directors of the National Bank of Commerce, New York, has appointed the following persons to the various committees of the Board for the year ending December 31, 1910:

The Finance Committee consists of the following members:

The Audit Committee consists of the following members:

The Executive Committee consists of the following members:

The Nominations Committee consists of the following members:

The Resolutions Committee consists of the following members:

Executive Committee

The Executive Committee of the National Bank of Commerce, New York, consists of the following members:

The Executive Committee has the honor to acknowledge the receipt of the following communication from the Board of Directors of the National Bank of Commerce, New York, dated December 15, 1910:

The Board of Directors of the National Bank of Commerce, New York, has appointed the following persons to the various committees of the Board for the year ending December 31, 1910:

The Finance Committee consists of the following members:

The Audit Committee consists of the following members:

The Executive Committee consists of the following members:

The Nominations Committee consists of the following members:

The Resolutions Committee consists of the following members:

for hexose sugars; a second characterized by a starvation type of growth at a low concentration and a slight increase in both growth and fruiting at higher concentrations; and a third or intermediate type in which growth and fruiting were poor at low concentrations but increased with an increase in carbohydrates until both were good. The type of response of three fungi was correlated with the rate at which sugar was inverted and with the amount of invertase produced (35).

In a later paper (33) it was established that certain di- and polysaccharides are better sources of carbon for fruiting of Melanospora destruens than the hexose sugars. While Miss Hawker considered that this was partly due to a rate of hydrolysis giving favorable concentration of hexoses over a relatively long period, she also presented evidence to show that the readiness with which certain phosphoric esters - e.g. glucose - 1 - phosphate are formed is significant. This worker also suggested that the amount of available energy may be a controlling factor.

Recently, Miss Hawker (34) has proved that phosphoric esters are beneficial to the sporulation of Melanospora. Perithecial production in a synthetic medium was stimulated by the addition of various hexose phosphates.

The first part of the report is devoted to a general survey of the situation in the country. It is followed by a detailed account of the work done during the year. The report then discusses the results of the work and the conclusions reached. Finally, it contains a list of references and a list of names of the persons who have assisted in the work.

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The third part of the report is devoted to a detailed account of the work done during the year. It is divided into several sections, each dealing with a different aspect of the work. The first section deals with the general survey of the situation in the country. The second section deals with the detailed account of the work done during the year. The third section deals with the results of the work and the conclusions reached. Finally, the fourth section contains a list of references and a list of names of the persons who have assisted in the work.

The stimulatory effect of glucose - 1 - phosphate, of a mixture of monophosphates, and of fructose - 1: 6 - diphosphate were approximately the same.

Westergaard and Mitchell (83) found that glucose or carbohydrates containing glucose are the most effective of the carbohydrates tested for inducing sporulation of Neurospora. High concentrations of sugar were superior to low concentrations.

Natural Products, Growth Factors, and Vitamins

Many fungi require media containing some material of natural or organic origin for growth and reproduction. Sometimes these natural products may be replaced by traces of vitamins or other growth factors.

Robbins and Kavanagh (69) have reviewed the effect of vitamins and growth factors on the development of the filamentous fungi, including sporulation. The phenomena of sexuality of the fungi in relation to these same substances has been discussed by Schopfer (75).

Snyder and Hansen (80) have described the superior value of pea straw agar sterilized by means of fumigation, over heat sterilized media, for the sporulation of Alternaria solani and a Gloeosporium sp.

Leaf juice expressed from Plantanus racemosa leaves and sterilized by filtration through Chamberlain filters was used successfully by Smith and Smith (79) for producing sporulation of four leaf-spotting fungi attacking this plant.

Mrak et al (58) used ground, unpeeled carrots, beets, cucumbers, and potatoes incorporated into a stock medium to induce fruiting of yeasts and other fungi.

Leonian (51) found that malt extract was necessary for reproduction of Valsa leucostoma. According to Shu and Johnson (77) this substance also accelerates spore formation by Aspergillus niger. Deschiens (18) was able to obtain a luxuriant yield of spores by growing certain Hyphomycetes on media containing oat chaff as well as malt extract.

Corticium rolfsii fruited abundantly on onion-proteose peptone and potato dextrose agar when Milthorpe (57) tested these media but the former was the better of the two. Venkatakrishtnaia (82) also succeeded in getting this organism to fruit using an onion asparagine agar.

Benham (6) reported recently that 49 of the 50 strains of Trichophyton rubrum tested, produced typical microconidia on blood agar base, whereas they were formed

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by only two of the strains when grown on Sabouraud's honey or dextrose agar. This author suspected that tryptose was the ingredient responsible for spore formation.

Working with Neurospora, Butler et al (8) concluded that biotin is essential for the growth but that other factors are necessary for free production of ascospores. However, Westergaard and Mitchell (83) reported recently that many strains of this organism fruit in a synthetic medium containing biotin. But certain mutants failed to produce perithecia unless certain ingredients such as yeast extract, malt syrup, or acid-hydrolyzed casein had been added to the medium.

Products of natural origin necessary for sporulation may often be replaced by minute traces of one or more of the known vitamins. Asthana and Hawker (2) noted that malt extract was necessary for the fruiting of Melanospora destruens. Miss Hawker (31) later demonstrated that this organism and a number of other unrelated fungi sporulated readily in a medium containing a crude extract of lentils. Later it was shown that biotin was necessary for good growth and thiamin for the production of perithecia by some of these fungi including Melanospora.

Nickerson and Thimann (59) discovered that the conjugation-promoting principle for certain Zygosaccharomyces species was produced by Aspergillus niger and that this principle could be replaced by a mixture of riboflavin and glutamic acid.

Hazen (36) observed that yeast extract is essential for growth and macroconidial formation of Microsporium audouini but that thiamin and pyridoxine could not substitute^{for} this substance.

Miller (56) reported that Venturia inaequalis and Sclerotinia fructicola gave highest yields of conidia on 10 percent malt agar. The best medium for fruiting of Phytophthora infestans was lima-bean agar. The addition of Brewer's yeast, yeast extract, riboflavin, thiamin chloride, and corn steep liquor in varying amounts to potato dextrose agar did not increase sporangial yield by the latter organism.

That biotin is essential for the sporulation of Sordaria fimicola has been shown by Barnett and Lilly (3). These same workers reported that Ceratostomella fimbriata requires thiamin for perithecial formation but possesses no deficiency for biotin, pyridoxine or inositol (4). They also found that

Chaetomium convolutum requires exogenous thiamin and biotin (54). Robbins and Ma (70) found that a Ceratostomella sp. requires thiamin. Recently, Leaver et al (50) have discovered that biotin and thiamin are required for growth and sporulation of Piricularia oryzae.

Agar may contain substances which induce sporulation of certain fungi. Robbins (67) demonstrated that agar contains a growth factor necessary for zygote formation by Phycomyces blakesleeanus. This substance was not replaceable by several of the known vitamins but was found to consist of two factors called Z₁ and Z₂. Factor Z₁ has been identified as hypoxanthine. The same activity is exhibited by guanine. Factor Z₂ remains unidentified (75).

Agar is also known to contain thiamin and biotin. The subject is reviewed by Day (17). She found that different kinds of agar varied in their thiamin content. Difco granulated agar contained about 0.1 ^{*}μg. of thiamin per gram and crude shredded agar about 0.5 μg. per gram. Robbins (68) reported that the biotin content of agar also varies with the sample amounting to approximately 0.1 μg. per gram in some cases.

* μg. = microgram = 0.001 mgn.

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Nutrient Ratios

The ratio of the concentration of one nutrient to another in the medium seems to influence the sporulation of some fungi. According to Schopfer (75) definite quantities of carbon (maltose) and of nitrogen (asparagine) are necessary for the production of zygotes by Mucor hiemalis. A definite ratio between these two constituents is also required. Above or below a certain ratio, zygotes do not develop.

Recently Barnett and Lilly (3) have emphasized the importance of the vitamin nutrient ratios. They suggest that a proper balance between the amount of biotin, the supply of nutrients, pH, and other factors are prerequisites for normal sexual reproduction in Sordaria fimicola (3). These workers also found that sexual reproduction by Ceratostomella fimbriata occurs only when the ratio of thiamin to the amount of nutrients in the medium is relatively high (4). Similarly for Chaetomium convolutum, the higher the concentration of food in the medium, the greater the amount of biotin and thiamin required for the formation of perithecia (54).

Leonian and Lilly (52) concluded that high or low dextrose-aspartic acid ratios tended to reduce zygote production by Phycomyces blakesleeanus.

On the other hand, Asthana and Hawker (2) demonstrated that sporulation of Melanospora depended on the actual amount of sugar and nitrogen present rather than on the ratio of these two.

Camp (9) observed that fruiting in the slime mold, Physarum polycephalum may be induced by exhaustion of nutrients.

Sporulation of Pyronema confluens begins in fluid cultures according to Robinson (71) only when the available nitrogen is becoming exhausted. Similarly no general development of reproductive structures occurs if the initial concentration of maltose is fairly high. Vegetative growth continues until the concentration of nitrogen and carbon is reduced to a level at which perithecia can be formed.

pH

Hydrogen-ion concentration of the medium may influence the sporulation of fungi. Leach (49) correlated abundant perithecial production by Mycophaerella musicola, the cause of banana leaf spot, with the growth of the host in highly acid soil of pH 4.0 to 4.75. Lilly and Barnett (53) reported that Sordaria fimicola would produce perithecia

... (faint text) ...

... (faint text) ...

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... (faint text) ...

in the presence of adequate growth factors only after the pH of the substrate had risen above 6. Similarly, Gardner (22) associated sporulation and elongation of conidiophores of Aspergillus gigantens with pH changes from 4.8 to basic. Anderson (1) found that the sporulation of Gibberella zeae was directly influenced by the initial pH which in time was dependent on the nitrogen source. On the other hand, Asthana and Hawker (2) observed that pH had no effect on sporulation by Melanospora within wide limits.

The Nutrition of O. graminis

Very few studies have been made of the nutrition of O. graminis in relation to growth and fruiting. Fellows (21) concluded that this organism is unable to utilize inorganic forms of nitrogen. Padwick (61) found that a nitrogen-free carrot extract contained a substance capable of stimulating growth in a synthetic nutrient solution. Garrett (24) later observed that O. graminis grew well if a wheat straw extract was provided in place of the carrot extract.

White (85) demonstrated that O. graminis requires biotin and thiamin for mycelial development. Growth occurs only when biotin is present; but more than double the amount of growth results when thiamin is also added. He reported

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that about 1000 μ g. each of biotin and thiamin are required for optimal growth conditions. Growth factors are present in optimal amounts in extracts of wheat roots, wheat straw, and peptone. The necessity of biotin and thiamin for mycelial development has been supported in a recent paper by Davies et al (15).

The work of Padwick (61), Garrett (24), and White (85), revealed that O. graminis may utilize various inorganic forms of nitrogen in the presence of growth factors and inorganic nutrients and disproved Fellow's earlier work which was conducted in the absence of growth factors. Later White (86) demonstrated that a wide range of compounds are utilized by this organism as a source of carbon and nitrogen for respiration and assimilation. He reported that the optimal concentration for the nitrogen source is equivalent to 200 mgs. nitrogen per litre and the carbon source is equivalent to 2.0 percent glucose when nitrogen is supplied as KNO_3 , NH_4NO_3 , glycine, or asparagine; but the concentrations are 200 mgs. of nitrogen per litre and 1.0 percent glucose when nitrogen is supplied as a mixture of amino acids or peptone.

White (86) also observed that there are differences in assimilability of compounds by O. graminis. When carbon and nitrogen are present in optimal amounts these differences are due to anabolite efficiency values of the compounds

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which condition the growth rate rather than the maximal amount of growth of the fungus and this (in addition to the biotin and thiamin requirement) is a third factor limiting the growth of O. graminis in synthetic solutions. The efficiency values are optimal for growth when nitrogen is supplied as a mixture of amino acids or peptone, and carbon is supplied as glucose in optimal amounts. White (86) conducted his experiments in liquid media and made no reports on the effect of various nutrients on sporulation.

Various natural media have been used successfully for inducing sporulation of this organism. Kirby⁽⁴⁷⁾ obtained perithecia on crushed wheat agar and sterilized sweet clover and wheat stems. Davis (16) found that string bean agar was a suitable medium. Of several media tested by Russell (72), ground oat hulls was the only satisfactory substrate for perithecial formation.

The first part of the report deals with the general situation of the country and the progress of the work done during the year. It also contains a list of the names of the members of the committee and of the persons who have assisted them in their work.

The second part of the report deals with the work done during the year. It is divided into two sections, the first of which deals with the work done in the field and the second with the work done in the office.

The third part of the report deals with the financial statement for the year. It contains a list of the items of income and expenditure and a statement of the balance sheet at the end of the year.

The fourth part of the report deals with the general remarks of the committee. It contains a list of the recommendations of the committee and a statement of the reasons for these recommendations.

The fifth part of the report deals with the conclusions of the committee. It contains a list of the conclusions of the committee and a statement of the reasons for these conclusions.

The Effect of Various Nutritional Factors on the
Development of O. graminis

Introduction

The preceding literature review reveals the importance of nutrition in the sporulation of fungi. As mentioned previously, Davies (14) had found a bacterium which would induce sporulation of O. graminis in pure culture. Since certain microorganisms are known to stimulate sporulation of other fungi by supplying them with required nutrients, it is possible that Davies' bacterium was able to supply O. graminis with some specific nutrient for sporulation. Thus the following studies were conducted in an attempt to determine the nutritional conditions necessary for the sporulation of O. graminis in artificial culture.

Methods

Strain 8 of O. graminis was used throughout these experiments. At times S₁ served as a check.

Unless otherwise stated, the basic nutrient solution had the following composition:

THE STATE OF TEXAS, COUNTY OF DALLAS, ss. I, _____, Clerk of the County, do hereby certify that _____ is the owner of _____

Witness my hand and seal of office this _____ day of _____, 19____.

Clerk of the County

Notary Public

Dextrose	20gm.
KH_2PO_4	1gm.
Na_2HPO_4	3gm.
MgSO_4	2gm.
NaNO_3	2gm.
FeCl_3	trace (0.001 gm)
NaBO_2	trace
CuSO_4	trace
ZnSO_4	trace
$(\text{NH}_4)_2\text{M}_2\text{O}_4$	trace
Agar	15gm.
Distilled water to make	1000 ml.

Other substances were added according to the treatment. The sources of the chemicals used in these studies were as follows:

sugars - Difco, Eastman Kodak, and Baker's; amino acids - Smaco and Eastman Kodak; thiamin hydrochloride and nicotinic acid - Merck; biotin, p-amino benzoic acid, and folic acid - General Biochemicals; various extracts and peptone - Difco.

All glassware was washed thoroughly with trisodium phosphate or dicromate-sulfuric acid cleaning solutions. Experiments were conducted in Petri plates, test-tubes or

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Erlenmeyer flasks. Each plate or flask contained 25 ccs. of medium while agar slants had 10 ccs. All media were sterilized at 120°C for 20 minutes. The pH was adjusted to 6.7 before autoclaving. The inoculum consisted of a bit of mycelium from a fresh culture growing on potato dextrose, basic nutrient, or water agars.

There were 5 to 10 replicates for each treatment. Some experiments were repeated 2 or 3 times. Cultures were incubated in a humidity chamber at 15° - 20°C, in diffuse light in the greenhouse. One experiment on growth alone was conducted in a 20°C constant-temperature chamber in the dark.

Ratings of growth were made at the end of 14 days. On agar these were based on the diameter of the colony and the density of growth. Development in liquid media was measured by the dry weight of mycelium. Examination for the presence of perithecia was made at the end of 8 weeks.

The Effect of Vitamins and Growth Factors

In this experiment, thiamin was added at the rate of 10000µg per litre of solution and other vitamins at

The first part of the report deals with the general situation of the country and the progress of the work done during the year. It is followed by a detailed account of the various projects and schemes which have been carried out, and a summary of the results achieved. The report concludes with a statement of the views of the Committee on the progress of the work and the prospects for the future.

The second part of the report deals with the financial position of the organization. It gives a detailed account of the income and expenditure for the year, and shows how the funds have been applied to the various projects and schemes. It also gives a statement of the assets and liabilities of the organization at the end of the year.

Statement of Accounts for the year ending 31st December 1954

The following table shows the income and expenditure of the organization for the year ending 31st December 1954.

the rate of 100 μ g. The extracts and peptone were supplied at a concentration of 20 gm. per litre.

The experiment was conducted in triplicate; with strains 8 and S₁ in Petri plates and with strain 8 in test tubes. Folic acid and p-amino benzoic acid were included only in the Petri plate study with strain 8. The inoculum was taken from cultures growing on basic nutrient agar.

The results of the studies with strain 8 are presented in Table VII and Figures 10 and 10a.

The necessity of biotin for growth and of thiamin for further vegetative development of O. graminis was confirmed. The further addition of niacin, riboflavin, calcium pantothenate, pyridoxine, folic acid, p-amino benzoic acid inositol, and vitamin C singly and in combination had no obvious effect on growth. Sufficient growth substances are evidently supplied in malt, yeast, and string bean extracts and in peptone. Growth is slightly better in the presence of the extracts than with peptone. Mycelium in the presence of inositol was dark in color as contrasted to the white fluffy growth on the other media.

Some growth occurred on the basic medium and on water agar. Evidently O. graminis is able to derive enough

The first part of the report deals with the general situation in the country and the progress of the work of the various departments. It is followed by a detailed account of the work of the different departments, and a summary of the results achieved. The report concludes with a number of recommendations for the future.

The work of the different departments has been carried out in accordance with the programme of work approved by the Council of Ministers. The results achieved are as follows:

The Ministry of Education has continued its work on the reform of the educational system. It has succeeded in introducing a number of important reforms, and in improving the quality of education. The Ministry of Health has also made considerable progress in its work. It has succeeded in reducing the incidence of many of the most common diseases, and in improving the health of the population.

The Ministry of Agriculture has also made considerable progress in its work. It has succeeded in increasing the production of many of the most important agricultural products, and in improving the living conditions of the rural population. The Ministry of Industry has also made considerable progress in its work. It has succeeded in increasing the production of many of the most important industrial products, and in improving the living conditions of the urban population.

The Ministry of Finance has also made considerable progress in its work. It has succeeded in increasing the revenue of the State, and in reducing the deficit of the State budget. The Ministry of Foreign Affairs has also made considerable progress in its work. It has succeeded in establishing good relations with many of the most important countries in the world, and in promoting the interests of the country in international affairs.

The results achieved by the different departments are a clear indication of the progress made by the country in the various fields of activity. It is hoped that these results will continue to be maintained, and that the country will continue to make progress in the future.

nutrients and growth factors from agar to produce some growth but not enough to cause discrepancy in the present studies.

Mature perithecia were not observed on any of the media. Perithecia-like black bodies were formed only when biotin and thiamin were both present. The further addition of pyridoxine, folic acid, and p-amino benzoic acid seemed to increase slightly the production of these bodies when added singly but not when added in combination with the other vitamins. Black bodies failed to form in the presence of malt and yeast extracts and peptone. Of all the substances tested, string bean extract seemed to be the most favorable for their production.

TABLE VII

THE EFFECT OF VARIOUS VITAMINS AND GROWTH FACTORS ON THE DEVELOPMENT OF O. GRAMINIS (STRAIN 8)

No.	Medium Description	Growth (14 days)		Relative number black bodies	Peri- thecia
		Diameter (cms.)	Density		
1	1.5% water agar	?	0.5	0	0
2	Basic	?	1	0	0
3	Basic + biotin	30.0	2.5	0	0
4	Basic + thiamin	?	1.5	0	0
5	Basic + biotin + thiamin	35.7	5	++	0
6	Medium 5 + niacin	38.4	5	++	0
7	5 + riboflavin	37.1	5	++	0
8	5 + calcium pantothenate	36.5	5	++	0
9	5 + pyridoxine	37.3	5	+++	0
10	5 + folic acid	38.0	5	+++	0
11	5 + p-amino benzoic acid	38.1	5	+++	0
12	5 + vitamin C	36.6	5	+++	0
13	5 + inositol	34.9	5	++	0
14	Basic + all vitamins above	36.9	5	++	0
15	Basic + 2% malt extract	34.4	9	0	0
16	Basic + 2% yeast extract	32.1	10	0	0
17	Basic + 2% string bean extract	33.2	10	++++	0
18	Basic + 2% peptone	36.3	8	0	0

? = Growth was so sparse that colony diameter could not be measured.

TABLE I

Summary of the results of the tests conducted on the various types of concrete specimens under the various conditions of temperature and humidity.

Specimen No.	Type of Specimen	Temperature (°C)		Relative Humidity (%)	Remarks	Strength Ratio (%)
		At Time of Test	At Time of Casting			
1	Normal	20	20	65	Control	100
2	Normal	20	20	65	Control	100
3	Normal	20	20	65	Control	100
4	Normal	20	20	65	Control	100
5	Normal	20	20	65	Control	100
6	Normal	20	20	65	Control	100
7	Normal	20	20	65	Control	100
8	Normal	20	20	65	Control	100
9	Normal	20	20	65	Control	100
10	Normal	20	20	65	Control	100
11	Normal	20	20	65	Control	100
12	Normal	20	20	65	Control	100
13	Normal	20	20	65	Control	100
14	Normal	20	20	65	Control	100
15	Normal	20	20	65	Control	100
16	Normal	20	20	65	Control	100
17	Normal	20	20	65	Control	100
18	Normal	20	20	65	Control	100
19	Normal	20	20	65	Control	100
20	Normal	20	20	65	Control	100

Notes: 1. All specimens were cast and cured under the same conditions. 2. The results are the average of three tests.

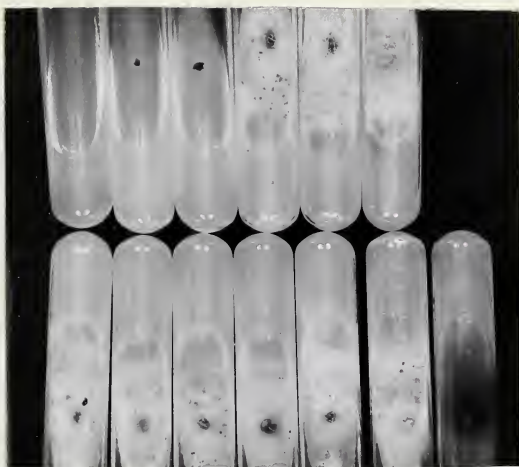


Figure 10

Effect of vitamins on the development of Ophiobolus graminis.

Upper row, left to right - water agar, basic nutrient medium, basic + biotin, basic + biotin + thiamin (medium 5), medium 5 + niacin, 5 + riboflavin.

Lower row, left to right - 5 + Ca. pantothenate, 5 + pyridoxine, 5+ folic acid, 5 + p-amino benzoic acid, 5 + vitamin C, 5 + mixture of vitamins, 5 + inositol.

Note the black bodies in several cultures and the dark colored mycelium in the presence of inositol.

Page 10

Statement of the Director of the Bureau of
Prisons

That the Bureau of Prisons is a part of the
Department of Justice, and is organized
under the direction of the Attorney General,
and is subject to the control of the
Department of Justice.

The Bureau of Prisons is organized into
several divisions, and is headed by
the Director of the Bureau of Prisons,
who is appointed by the President of
the United States.

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several divisions, and is headed by
the Director of the Bureau of Prisons,
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the United States.

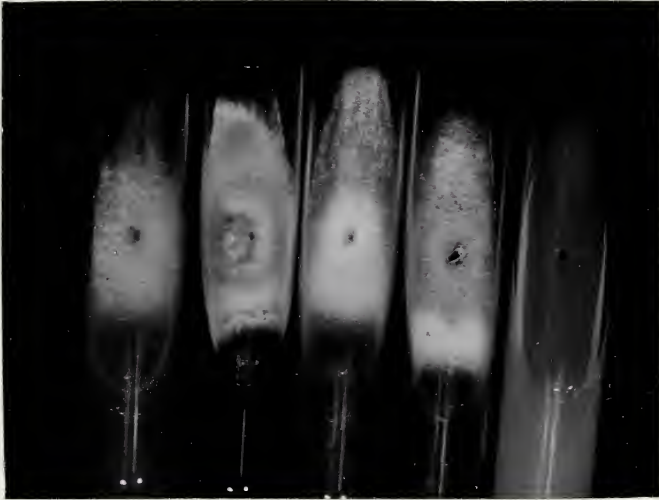


Figure 10a

Effect of growth factors on the growth of Ophiobolus graminis.

Left to right - Basic nutrient medium + peptone,
basic + string bean extract,
basic + yeast extract, basic +
malt extract, and basic alone.

SECRET

Approved by _____

The Effect of Concentration of Biotin and Thiamin

Inasmuch as only one concentration of biotin and thiamin was used in the previous experiment, it was decided to test the action of other concentrations. The basic medium was supplemented with various concentrations of these two vitamins and their effect on the development of O. graminis in Petri plates was noted and recorded in Table VIII. The effect of concentration of biotin and thiamin on growth is shown in Figures 11 and 12.

The results further illustrate the necessity of biotin for the growth of O. graminis. Growth was very poor on basic agar medium but there was fair development when biotin was present at the rate of 0.1 μ g. per litre. Although there was good growth when biotin was supplied in concentrations of 1.0 to 100 μ g. per litre, 100 μ g. seemed to provide optimum conditions. O. graminis was inhibited at a concentration of 1000 μ g. per litre.

Growth was much better when thiamin was also added to the medium. A concentration of 10,000 μ g. per litre seemed to induce maximum development. Concentrations greater than this had no further stimulatory effect.

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TABLE VIII

THE EFFECT OF CONCENTRATION OF BIOTIN AND THIAMIN
ON THE DEVELOPMENT OF O. GRAMINIS

Concentration ($\mu\text{g}/\text{l.}$)		Growth		Relative No. black bodies	Peri- thecia
Biotin	Thiamin	Diameter (cms.)	Density		
0	0	?	1	0	0
0	1000	18	3	0	0
0.1	1000	65	3.5	0	0
1.0	1000	77	7	+	0
10	1000	86	7	++	0
100	1000	87	8	+++	0
1000	1000	42	9	+++	0
100	0	68	7	0	0
100	100	75	8	+++	0
100	1000	87	8	++++	0
100	10,000	90	10	+++	0
100	100,000	88	10	+++	0
100	1,000,000	91	10	+++	0

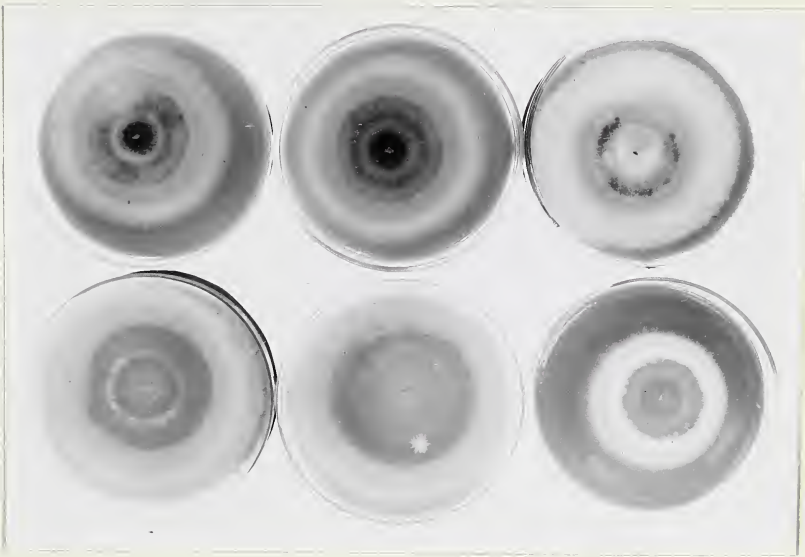


Figure 11

Effect of concentration of biotin on growth of Ophiobolus graminis.

Concentration of biotin in μg . per litre, left to right -

Upper row - 0, 0.1, and 1.0.

Lower row - 10, 100, 1000, (note inhibition).

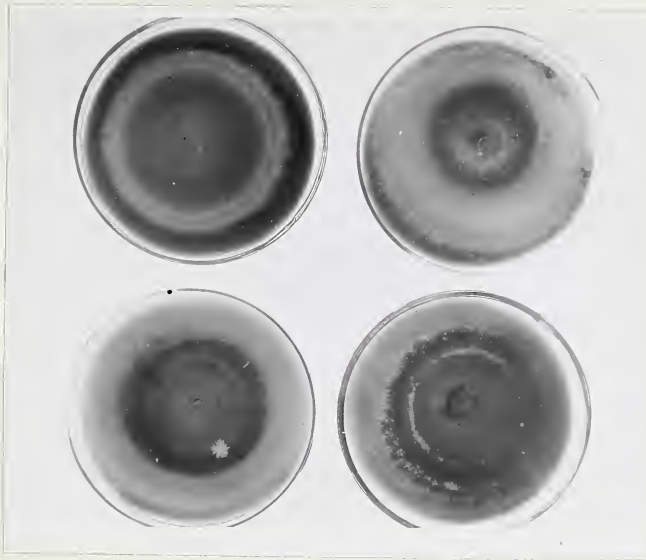


Figure 12

Effect of concentration of thiamin on the growth of
Ophiobolus graminis.

Concentration of thiamin in μ g. per litre, left to right -

Upper row - 0 and 100; lower row - 1000 and 10,000.

1948

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

REPORT OF THE COMMISSIONER OF AGRICULTURE
ON THE PROGRESS OF THE AGRICULTURE OF THE UNITED STATES
DURING THE YEAR 1948

No perithecia were observed at any concentration of biotin and thiamin. No black bodies were formed if thiamin was absent from the medium but increasing the concentration of biotin with thiamin present increased the relative number of these bodies produced. None were formed below a concentration of 1.0 μ g. of biotin per litre. Concentrations of biotin and thiamin above 100 μ g. per litre did not stimulate their formation further.

In a study of the effect of the interaction of outdoor conditions and concentration of vitamins on the development of O. graminis the above experiment was repeated placing the cultures outdoors where they were subjected to diffuse daylight and the alternating temperatures of midsummer. These conditions had no more effect on O. graminis than was observed in the previous greenhouse studies.

The Effect of Concentration of Nutrients

An attempt was made to determine the effect on the development of O. graminis of reducing the concentration of the nutrients while holding the concentration of the growth factors constant. A series of media were prepared with each of the following dilutions of nutrients: undiluted,

to the extent that the Commission is not satisfied

with the information provided by the applicant, the Commission may require the applicant to provide further information. The Commission may also require the applicant to provide information in a form and manner specified by the Commission. The Commission may also require the applicant to provide information in a form and manner specified by the Commission. The Commission may also require the applicant to provide information in a form and manner specified by the Commission.

in a form and manner specified by the Commission. The Commission may also require the applicant to provide information in a form and manner specified by the Commission. The Commission may also require the applicant to provide information in a form and manner specified by the Commission. The Commission may also require the applicant to provide information in a form and manner specified by the Commission.

The Effect of the Commission's Decision

to the extent that the Commission is not satisfied with the information provided by the applicant, the Commission may require the applicant to provide further information. The Commission may also require the applicant to provide information in a form and manner specified by the Commission. The Commission may also require the applicant to provide information in a form and manner specified by the Commission.

1/2, 1/4, 1/8, 1/16, 1/32, 1/64. Biotin and thiamin were added to each of these dilutions at the rate of 100 and 10,000 µg. per litre respectively. A bit of culture growing on water agar served to start the colonies in these Petri plate studies.

TABLE IX

THE EFFECT OF CONCENTRATION OF NUTRIENTS ON THE DEVELOPMENT OF O. GRAMINIS

Dilution of nutrients	Growth		Production of black bodies (8 weeks)	Peri-thecia
	Diameter (cms.)	Density		
0 (No vitamins)	tr	1	0	0
0 (Vitamins present)	80.5	10	++	0
1/2 "	74.0	6	++	0
1/4 "	67	2.5	0	0
1/8 "	62.5	1.5	0	0
1/16 "	63	very low	0	0
1/32 "	58.5	very low	0	0
1/64 "	51.5	very low	0	0

The following table shows the results of the tests conducted on the various samples of the material under investigation. The results are given in terms of the percentage of material which is soluble in water, and the percentage of material which is insoluble in water. The results are given in the following table:

Table

Results of tests conducted on various samples of the material under investigation.

Sample No.	Soluble in Water		Insoluble in Water	
	Percentage	Weight	Percentage	Weight
1	100	1.00	0	0.00
2	100	1.00	0	0.00
3	100	1.00	0	0.00
4	100	1.00	0	0.00
5	100	1.00	0	0.00
6	100	1.00	0	0.00
7	100	1.00	0	0.00
8	100	1.00	0	0.00

The results presented in Table IX show that diluting the nutrients greatly reduces the growth of O. graminis. Growth was poor if the concentration of nutrients was reduced to 1/4 of the original. Furthermore, black bodies were not formed below the 1/2 level. No perithecia were observed at any dilution.

The results also show that biotin and thiamin are ineffective as growth factors if adequate nutrients are not supplied. This suggests that the role of these two vitamins added in minute quantities is not in the manner of a nutrient but more in the form of a catalyst or some other similar agent acting on the nutrients in the medium in the production of substances necessary for the growth of the organism.

The Replacement of Biotin by Aspartic Acid

A number of reports have appeared indicating that biotin and aspartic acid are interchangeable as growth factors for certain fungi and bacteria. Beadle and Tatum (5) noted that aspartic acid reduces the biotin requirement of Neurospora and Schopfer (76) found that a similar relationship holds for Phycomyces blakesleeanus. Recently, a report

by Perlman (63) indicates that the addition of this same amino acid to a synthetic medium also markedly reduced the biotin requirement of Memoniella echinata. Furthermore, Koser et al (48) were able to demonstrate that for Torula cremoris, aspartic acid could replace biotin. Conversely, Stokes et al (81) observed that biotin can completely substitute for aspartic acid in the growth of Lactobacillus arabinosus, Streptococcus faecalis and related organisms. They concluded that biotin participates in the synthesis of aspartic acid. Recently, Potter and Elvehjem (64) have also noted that aspartic acid and biotin are almost interchangeable in the nutrition of L. arabinosus.

The ability of aspartic acid to substitute for biotin as a growth factor for O. graminis in the absence of thiamin was investigated in these studies. Aspartic acid at the rate of 1 gm. and biotin at the rate of 100, 500, and 1000 μ g. were added singly to a litre of basic nutrient medium. The experiment was conducted in liquid media in flasks. The results presented in Table X indicate that aspartic acid may almost completely substitute for the biotin requirement of this organism. They also support the previous observation that biotin at concentrations over 100 μ g. per litre may inhibit the growth of O. graminis.

The first part of the report deals with the general situation of the country and the progress of the war. It is followed by a detailed account of the military operations in the various theaters of war. The author then discusses the political and economic conditions of the country and the impact of the war on the population. The report concludes with a summary of the findings and a list of recommendations.

The following is a list of the main points discussed in the report:

- 1. General situation of the country and progress of the war.
- 2. Military operations in the various theaters of war.
- 3. Political and economic conditions of the country.
- 4. Impact of the war on the population.
- 5. Summary of findings and recommendations.

The report is a valuable source of information for anyone interested in the current situation of the country and the progress of the war. It provides a comprehensive overview of the various aspects of the conflict and its impact on the population.

TABLE X

UTILIZATION OF BIOTIN AT VARIOUS CONCENTRATIONS AND ASPARTIC ACID AS GROWTH SUBSTANCES BY O. GRAMINIS

Medium	Dry weight of mycelium in mg. (21 days)
Basic	9.9
Basic + Biotin (100 μ g/l.)	47.4
Basic + Biotin (500 μ g/l.)	43.8
Basic + Biotin (1000 μ g/l.)	41.4
Basic + Aspartic acid (1 g/l.)	36.4

Carbon Source

Since the previous experiments were conducted using only dextrose as the carbon source and since perithecial formation was lacking in these cases, it was thought advisable to test the effect of other carbohydrates on the development of O. graminis. In this experiment, growth substances were supplied as wheat-stem extract which was prepared by autoclaving 200 grams of wheat stubble with a litre of water for 20 minutes. The resulting extract was decanted off, filtered, and made up to a litre volume with

STATE OF CALIFORNIA

STATE OF CALIFORNIA		REVENUE ACCOUNT	
FISCAL YEAR 1967-68		GENERAL FUND	
ACCOUNT	AMOUNT	AMOUNT	PERCENT
1.1			100%
1.2	1,200,000,000		100%
1.3	1,200,000,000		100%
1.4	1,200,000,000		100%
1.5	1,200,000,000		100%

STATE OF CALIFORNIA

THE STATE OF CALIFORNIA, by and through the Board of Equalization, do hereby certify that the foregoing is a true and correct copy of the report of the Board of Equalization for the fiscal year 1967-68, as required by the provisions of the Constitution of the State of California, Article XIII, Section 1, and the provisions of the Revenue and Taxation Code, Section 10000.

IN WITNESS WHEREOF, I have hereunto set my hand and the seal of the State of California, at Sacramento, California, this 1st day of January, 1968.

GOVERNOR

water. This extract replaced the water in the basic nutrient medium which was otherwise unchanged except for the carbon source.

TABLE XI

THE EFFECT OF VARIOUS CARBON SOURCES ON THE DEVELOPMENT OF O. GRAMINIS

Carbon source	Growth rating (14 days)	Relative No. black bodies (8 weeks)	Perithecia
dextrose	8	0 ¹	0
starch	7	0 ¹	0
xylose	7	0 ¹	0
inulin	2	0	0
maltose	10	0 ¹	0
d-mannose	8	0	0
laevulose	8	0 ¹	0
d-galactose	6	0 ¹	0
lactose	7	0 ¹	0
sucrose	6	0 ¹	0
None	1	0	0

1 = Few poorly developed black bodies.

THE UNIVERSITY OF CHICAGO
DEPARTMENT OF CHEMISTRY
5500 S. UNIVERSITY AVENUE
CHICAGO, ILLINOIS 60637

RECORD

NAME OF STUDENT: _____
DATE OF EXAMINATION: _____

Q. NO.	ANSWER	MARKS	TOTAL MARKS
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
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THE UNIVERSITY OF CHICAGO

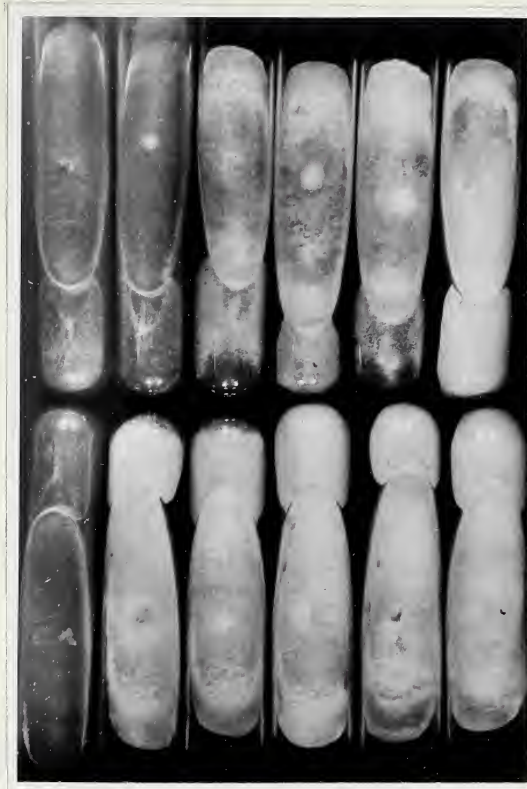


Figure 13

Effect of carbon source on the growth of Ophiobolus graminis.

Carbon source added to basic nutrient medium with wheat-stem extract as the growth factor source, left to right -

Upper row - check, inulin, sucrose, d-galactose, xylose, starch.

Lower row - check, lactose, dextrose, d-mannose, laevulose, maltose.

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Table XI and Figure 13 show that maltose was the best carbon source for the growth of O. graminis at the end of 14 days and inulin was the poorest source. Dextrose, d-mannose and laevulose were intermediate and induced better growth than starch, xylose, d-galactose, lactose and sucrose.

No perithecia or fully-formed black bodies developed on any of the media. However, with several carbon sources, small black specks appeared in the cultures. These may be similar to the perithecia-like black bodies formed in the previous experiments but at a more immature stage of development.

Concentration of Dextrose

In all previous experiments, dextrose and the other carbon sources were supplied at the rate of 2% by weight of the medium. Previous work by White (86) had shown that this was the optimum level for growth of the organism in the presence of inorganic nitrogen. To test the effect of other concentrations of dextrose on the general development of O. graminis this sugar was added in varying quantities to the basic nutrient and wheat-stem

agar medium used in the previous experiment. This test was made using agar slants inoculated with a culture from basic nutrient agar.

TABLE XII

THE EFFECT OF CONCENTRATION OF DEXTROSE ON THE DEVELOPMENT OF O. GRAMINIS

<u>% concentration of dextrose</u>	<u>Growth rating (14 days)</u>	<u>Relative no. black bodies (8 weeks)</u>	<u>Perithecia</u>
0	1	0	0
0.5	6	0	0
1.0	7	0	0
1.5	7	0	0
2.0	8	0 ¹	0
5.0	7	++	0

¹ = Few poorly developed black bodies.

The results presented in Table XII and illustrated in Figure 14 show that dextrose at the rate of 2% is optimum for mycelial development but that a wide range of concentrations induce satisfactory growth under the conditions of this test.

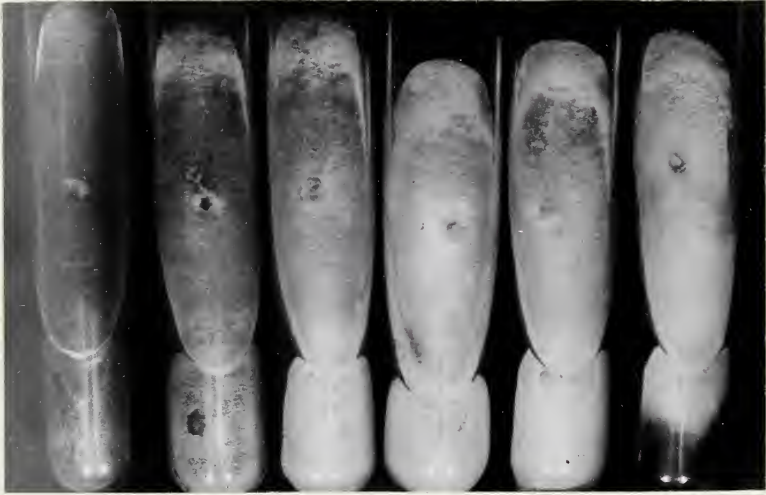


Figure 14

Effect of concentration of carbon source (dextrose) on
the growth of Ophiobolus graminis

Concentration of dextrose in percent,
left to right - 0, 0.5, 1.0, 2.0, and 5.0.

The following is a list of the names of the persons who have been
admitted to membership in the Society since the last meeting.
 The names are given in the order in which they were admitted.
 J. A. B. C. D. E. F. G. H. I. J. K. L. M. N. O. P. Q. R. S. T. U. V. W. X. Y. Z.

With a concentration of 5% dextrose, typical perithecia-like bodies similar to those formed in other experiments appeared in great numbers in the cultures by the end of 8 weeks. As in the previous experiment tiny black specks developed at the 2% level of dextrose but none were observed below this concentration. These results show that concentration of carbon source may greatly affect the development of these perithecia-like bodies. These bodies which developed at the 2% level of dextrose on this medium where growth substances were supplied as wheat-stem extract may be similar to those appearing at the 5% level on the same medium and at the 2% level on media containing biotin and thiamin. They are possibly at an earlier stage of development. This possibility was not investigated further.

Nitrogen Source

As previously reported by White (86) the anabolite efficiency values for O. graminis are optimal for growth when nitrogen is supplied as a mixture of amino acids or peptide, and carbon as glucose at optimal concentrations. In the light of the foregoing and inasmuch as NaNO_3 was the

nitrogen source in all of the experiments reported thus far it was thought advisable to investigate further the effect of various other nitrogen sources on the development of this fungus.

NaNO_3 was replaced by several nitrogen sources at the rate of 0.2% by weight in the basic medium. Biotin and thiamin were added at the rate of 100 and 1,000 μg . per litre respectively. Test-tube slants containing the various media and prepared in the usual manner were inoculated with basic nutrient agar cultures. The results are presented in Table XIII and Figure 15.

Under the conditions of this experiment, peptone and asparagine were the best sources of nitrogen for growth of O. graminis at the end of 14 days. A mixture of 13 amino acids was more effective than any of the individual acids used alone with the exception of d-glutamic acid. The poorest nitrogen source tested was dl-threonine. NaNO_3 was intermediate in effect. These results disagree slightly with the findings of White (86) who reported that peptone or an amino acid mixture was superior to asparagine as a nitrogen source.

No perithecia were produced in any of the media. NaNO_3 and dl-serine were the only nitrogen sources which induced the production of the perithecia-like black bodies.

TABLE XIII

THE EFFECT OF VARIOUS NITROGEN SOURCES ON THE
DEVELOPMENT OF O. GRAMINIS

Nitrogen source	Growth rating (14 days)	Relative no. black bodies (8 weeks)	Perithecia
None (ck.)	1	0	0
NaNO ₃	5	++	0
asparagine	10	0	0
peptone	10	0	0
aspartic acid	5.0	0	0
l-tryptophane	3.0	0	0
methionine	4.0	0	0
l-histidine	6.0	0	0
phenylalanine	4.5	0	0
dl-isoleucine	3.5	0	0
dl-threonine	2.0	0	0
d-arginine	7.0	0	0
dl-valine	4.5	0	0
l(-)cystine	2.0	0	0
d-glutamic acid	8.0	0	0
l-proline	5.5	0	0
dl-serine	5.5	++	0
Above 13 amino acids in combination	7.5	0	0

Table 1

Summary of the results of the analysis of variance for the different factors

Source of variation	D.F.	Mean square	F-value	Significance
Replication	1	1.20	0.05	n.s.
Treatment	5	1.80	0.75	n.s.
Block	10	0.50	0.20	n.s.
Error	40	0.25		
Total	56			

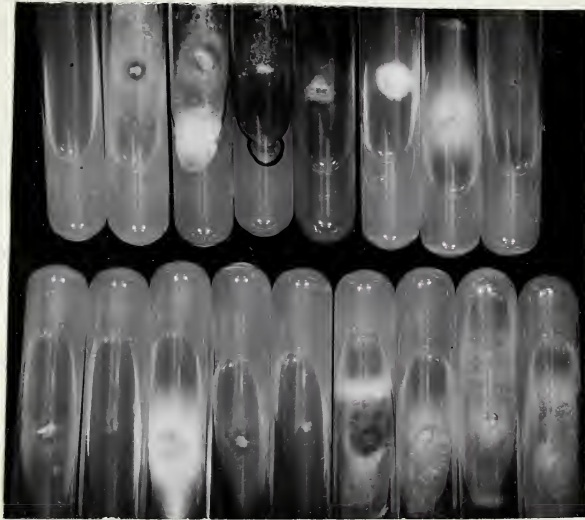


Figure 15

Effect of nitrogen source on the growth of Ophiobolus
graminis.

Nitrogen source, left to right -

Upper row - check, NaNO_3 , asparagine, peptone,
l-proline, methionine, phenylalanine,
dl-isoleucine.

Lower row - l-tryptophane, l-cystine, l-histidine,
dl-serine, dl-threonine, d-glutamic acid,
dl-valine, d-arginine, mixture of amino
acids.

TABLE II

Summary of the results of the tests of the various types of ...

1. ...

2. ...

3. ...

pH

It has been well established that O. graminis will grow in synthetic solutions over a wide range of pH. However, since this factor may influence the sporulation of other fungi, it may also play a part in perithecial production by O. graminis. This possibility was investigated in the following experiment.

Potato dextrose agar was adjusted with HCl or NaOH to various pH levels. Readings were made with a Beckman glass electrode pH meter before and after sterilization and after the fungus had grown on the media for 6 weeks. These readings are presented in Table XIV.

The pH values of acid media increased as a result of autoclaving while those of basic media were lowered. Hydrogen-ion concentration also tended toward neutrality under the action of the growing organism.

A wide range of initial pH values had no deleterious effect on growth of O. graminis. Neither perithecia nor perithecia-like black bodies were observed at any level. These results support the conclusions of other workers that pH has no effect on the development of O. graminis over a wide range.

The first part of the document is a list of names and titles, including the names of the authors and the titles of their works. The list is arranged in a specific order, and each entry is followed by a brief description of the work.

The second part of the document is a list of names and titles, including the names of the authors and the titles of their works. The list is arranged in a specific order, and each entry is followed by a brief description of the work.

The third part of the document is a list of names and titles, including the names of the authors and the titles of their works. The list is arranged in a specific order, and each entry is followed by a brief description of the work.

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The tenth part of the document is a list of names and titles, including the names of the authors and the titles of their works. The list is arranged in a specific order, and each entry is followed by a brief description of the work.

TABLE XIV

THE EFFECT OF GROWTH OF *O. GRAMINIS* AND
HEAT STERILIZATION ON THE pH OF
POTATO DEXTROSE AGAR

Initial pH	pH after sterilization	pH after 6 weeks growth of <i>Ophiobolus</i>
3.1	4.5	6.0
4.4	5.4	6.0
6.0	6.0	6.1
7.5	7.5	6.8
9.0	8.4	6.8

Discussion

The foregoing studies on the effect of various nutritional factors on the development of *O. graminis* in synthetic culture have provided definite information on the growth requirements of this organism. It was found that biotin is essential for growth and thiamin for further development thus supporting the previous observations of White (85) and Davies et al (15). Several substances of

TABLE I

Summary of the results of the tests conducted on the various types of concrete under various conditions of curing and loading

Designation of concrete	Modulus of elasticity	Strength	Modulus of rupture
1.0	1.5	1.0	1.0
2.0	2.0	1.5	1.5
3.0	2.5	2.0	2.0
4.0	3.0	2.5	2.5
5.0	3.5	3.0	3.0

CONCLUSIONS

The results of the tests conducted on the various types of concrete under various conditions of curing and loading are summarized in Table I. It is seen that the modulus of elasticity, strength, and modulus of rupture of the concrete increase with the curing time and the loading conditions. The increase in strength and modulus of rupture is more pronounced than the increase in modulus of elasticity. The results of the tests conducted on the concrete under various conditions of curing and loading are summarized in Table I.

plant or animal origin may satisfy this requirement. Even agar seems to supply small amounts of these growth factors.

The inhibiting effect of biotin at the concentration of 1000 μ g. per litre observed in these studies does not agree with White's observation that this concentration was optimum for growth. Possibly the different physical conditions in the liquid media of White and the solid media of most of the present studies may account for this disagreement. However, one experiment reported here suggests that there is also inhibition at 1000 μ g. per litre in liquid media. It is interesting to point out here that Schopfer (75) reported that excessive dosages of thiamin inhibited the growth of certain Rhizopus spp. but that this was dependent on certain physical conditions of the culture and that one nitrogen source prevented or retarded this inhibiting action.

Increasing the concentration of thiamin above a certain level had no further stimulatory effect on growth of O. graminis but caused no inhibition. These contrasting effects of high concentrations of biotin and thiamin coupled with the fact that biotin must be provided before thiamin is effective, suggests that these two substances have different functions in the physiological activity of O. graminis and that the function of the thiamin is dependent on the presence of biotin.

According to Schopfer (75) thiamin is an essential constituent of coenzymes. The loss of the ability to synthesize thiamin thus means that vital enzymatic reactions cannot be carried out unless this vitamin is supplied as an exogenous growth factor. Recently the role of biotin has been linked up with the production of aspartic acid by microorganisms. It is interesting that aspartic acid may partially replace the biotin requirement of O. graminis. Further studies are necessary before the relationship between biotin, thiamin, and aspartic acid in the physiology of this organism, may be understood.

It is unlikely that the ability of aspartic acid to replace the biotin requirement of Ophiobolus is a characteristic of many amino acids. Fellows (21) tested the effect of several amino acids including aspartic acid on the growth of O. graminis in a synthetic medium similar to that used here but lacking biotin. None of the amino acids tested could induce growth.

Several other vitamins are unable to further stimulate growth of O. graminis when biotin and thiamin are present at optimum concentrations. However, yeast, malt, and string-bean extracts and peptone are able to replace these two growth factors and induce more growth than

The first part of the report is devoted to a general
 description of the work done during the year. It
 contains a list of the projects undertaken and a
 summary of the results obtained. The second part
 is devoted to a detailed description of the work
 done on the project entitled "The effect of
 temperature on the rate of reaction between
 hydrogen peroxide and potassium iodide". This
 project was carried out by Mr. J. H. Smith and
 Miss A. B. Jones. The results of their work are
 given in the following table:

Temperature (°C)	Rate of reaction (moles/litre/second)
10	0.0012
20	0.0024
30	0.0048
40	0.0096
50	0.0192

It will be seen from the above table that the
 rate of reaction increases with temperature.
 This is to be expected, since the rate of
 reaction is known to increase with temperature.
 The results obtained are in good agreement with
 the theoretical predictions.

The third part of the report is devoted to a
 description of the work done on the project
 entitled "The effect of concentration on the
 rate of reaction between hydrogen peroxide and
 potassium iodide". This project was carried out
 by Mr. J. H. Smith and Miss A. B. Jones. The
 results of their work are given in the following
 table:

Concentration (moles/litre)	Rate of reaction (moles/litre/second)
0.1	0.0012
0.2	0.0024
0.3	0.0036
0.4	0.0048
0.5	0.0060

It will be seen from the above table that the
 rate of reaction increases with concentration.
 This is to be expected, since the rate of
 reaction is known to increase with concentration.
 The results obtained are in good agreement with
 the theoretical predictions.

The fourth part of the report is devoted to a
 description of the work done on the project
 entitled "The effect of catalyst on the rate of
 reaction between hydrogen peroxide and potassium
 iodide". This project was carried out by Mr.
 J. H. Smith and Miss A. B. Jones. The results
 of their work are given in the following table:

Time taken for reaction to complete (seconds)	Concentration (moles/litre)
100	0.1
50	0.2
25	0.3
15	0.4
10	0.5

It will be seen from the above table that the
 time taken for the reaction to complete decreases
 as the concentration increases. This is to be
 expected, since the rate of reaction is known
 to increase with concentration. The results
 obtained are in good agreement with the
 theoretical predictions.

occurs when the vitamins are present in optimum concentrations. White (85) observed the same phenomenon and later demonstrated (86) that this was the result of the anabolite efficiency values of these compounds which increases the growth rate rather than the maximal amount of growth of the fungus.

Inulin is a poor source of carbon for the growth of O. graminis which agrees with Miss Hawker's observations on the relationship between this substance and a starvation type of growth by Melanospora destruens. Best growth of O. graminis was obtained with maltose but all of the sugars tested induced good growth.

The growth reaction of O. graminis to different concentrations of dextrose observed here is in contrast to the reaction of several Ascomyetes to glucose and fructose as observed by Hawker and Chaudhuri (35). O. graminis grows well over a wide range of concentrations (0.5 - 5.0 percent) whereas growth of their organisms increased with increase in hexose sugars up to a concentration of 10 percent or more.

White (86) pointed out that the growth rate of O. graminis varied according to the complexity of the nitrogen compound (the more complex the compound the greater the rate of growth)

but the maximal growth was approximately the same in all substrates. He noted that 12 to 14 days was needed for maximum development with peptone and asparagine, 17 days with glycine, and 23 days with NaNO_3 . Furthermore, White suggested that O. graminis probably has an unspecialized nutrition in relation to a supply of nitrogen. Considering the present studies in the light of White's observations one would expect that all of the amino acids tested would induce better growth than NaNO_3 at the end of 14 days since they are more complex nitrogen sources. But this is not the case for l-tryptophane, methionine, phenylalanine, dl-threonine, dl-valine, l(-)cystine and dl-serine. This would indicate that for growth O. graminis may utilize certain nitrogen sources much better than others. It would be unwise to draw definite conclusions from the results of the single experiment reported here; yet until further information is provided on this matter the suggestion that O. graminis has an unspecialized nutrition in relation to nitrogen requirements for growth should be considered with caution.

In these studies none of the nutritional conditions provided induced ascospore formation by O. graminis. This would suggest that sexual reproduction of this organism is dependent on some other factor or combination of factors on a highly specific nutritional requirement not provided here.

The following is a list of the names of the persons who have been
 named in the report of the committee on the subject of the
 proposed amendment to the constitution of the State of New York.
 The names are given in the order in which they were mentioned
 in the report. The names of the persons who were named in the
 report are given in italics. The names of the persons who were
 not named in the report are given in plain type. The names of
 the persons who were named in the report and who were also
 named in the report of the committee on the subject of the
 proposed amendment to the constitution of the State of New York
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 constitution of the State of New York are given in plain type.

If the perithecia-like black bodies formed throughout these studies are considered to have any relationship to true perithecia, it is logical to conclude that O. graminis has certain specific nutritional requirements for perithecial formation. Production of the black bodies was conditioned by the presence in the culture medium of certain specific growth factors at definite concentrations. Their occurrence was also dependent on the presence of certain specific nitrogen sources and definite concentrations of nutrients.

There is the possibility that some of the nutrients used in the present studies were ineffective in inducing sporulation because they were inactivated by the sterilizing process of heating media for 20 minutes at 120°C. Hawk et al (30) state that thiamin, biotin, nicotinic acid, pyridoxine hydrochloride, folic acid and pantothenic acid are all stable when so treated. Patton and Hill (62) have recently shown that the autoclaving of media containing glucose may inactivate to some extent the B complex vitamins and certain amino acids. The latter include lysine, arginine, and tryptophane. Riesen et al (66) observed that sterilization by autoclaving had varying destructive effects upon the activity of cystine and cysteine,

depending upon the organism. These latter workers report that most amino acids added prior to the autoclaving process may be recovered satisfactorily. It seems unlikely from the foregoing reports that the sterilization process would have any serious inactivating effect on most of the substances used under the conditions of the experiments reported here. Thus inactivation probably does not account for the inability of these substances to induce sporulation of O. graminis in artificial culture in these studies.

GENERAL DISCUSSION

Previous to the initiation of these studies a bacterium had been found in this laboratory which would induce sporulation of O. graminis in pure culture (14, 15). Also Garrett (25) had suggested but had supplied no experimental proof that the other microorganisms in the soil might influence sporulation of this organism on the host. Other investigators felt that certain physical factors including light, temperature and moisture might play important roles. Furthermore it had been established that O. graminis is homothallic but that there remained the possibility that one strain might supply some stimulus to another strain in pure culture which might induce sporulation.

O. graminis sporulates fairly readily on the host plant if proper physical conditions are supplied. Possibly Davies' bacterium and the host plant are able to furnish O. graminis with substances necessary for sporulation. However, in the present studies, none of the vitamins, growth factors, nitrogen and carbon sources added to a basic nutrient medium in various concentrations and combinations was able to replace the effect of the host or Davies' bacterium. Assuming that the black bodies observed in these studies are immature

perithecia it is clear that nutrition plays a significant role in the sporulation of O. graminis.

These and other studies have demonstrated that many individual microorganisms are unable to induce sporulation of O. graminis in artificial culture. Either few individuals have the stimulating ability or pure culture conditions do not provide the proper relationship between the introduced organism and O. graminis for stimulation to occur. The same microorganism may have different effects on the growth of O. graminis at different stages of development. Since a similar relationship may hold sporulation this should receive further study.

Davies (14) found that only the living bacterium was able to induce fruiting of O. graminis in artificial culture. A sterile, staled liquid medium in which the bacterium had grown was ineffective. Possibly the bacterium must be supplied with some stimulation from O. graminis before it may be stimulatory itself. O. graminis might react to any stimulation at only a definite stage of development. More than one substance may be required in proper sequence for perithecial production. In the latter case, it is likely that only a few individual microorganisms would be able to meet such a demand. However, in nature all the conditions

suggested above might be supplied more easily by several microorganisms in association with O. graminis and account for the fact that this organism sporulates more readily in nature than in artificial culture. Davies et al (15) concluded that O. graminis sporulated more abundantly on wheat plants growing in unsterile soil than in sterile. This conclusion was not borne out by the present studies which suggested the importance of individual organisms. However, it should be appreciated that in such studies it is difficult to duplicate environmental conditions. Since the microflora of the soil varies greatly with the sample it is unlikely that the types and number of microorganisms present in any two experiments would be identical. Furthermore, discrepancies might arise from the biological variation of different individual plants and varieties of the host and of different strains of the fungus. Further studies are required before sufficient information will be available to explain the effect of other organisms on the sporulation of O. graminis. These should include the growing of two or more different organisms in association with O. graminis at the same time in an attempt to approach the more natural conditions of the soil.

These present studies supplied little information on the role that physical factors play in the sporulation

of O. graminis. Light is evidently important at least to the extent of playing a part in the maturation of perithecia. Definite information on the effect of the physical factors on sporulation will probably not be available until after a technique is devised for inducing sporulation on a medium other than the host plant. By present methods physical conditions acting on the microorganisms also act on the substrate which does not allow the investigator to hold all the conditions uniform while any individual physical factor is under study.

In the light of present knowledge, it seems definite that O. graminis is homotallic and that under the proper conditions single strains may sporulate in the absence of other strains. However, there is often a growth interaction between colonies of different strains in pure culture. Such an interaction might increase perithecial formation once it was initiated. This explanation could possibly account for Kirby's (46) conclusions that + and - strains must be grown together before sporulation of O. graminis will occur.

These studies have shown that independent factors may be important in inducing fruiting. However, none was found which governed the process. Sporulation might depend on a proper balance between several factors. The importance of different physical, nutritional and microbiological conditions in various relationships warrants further study.

The first part of the report deals with the general situation of the country and the progress of the work done during the year. It is followed by a detailed account of the various projects and schemes undertaken, and a summary of the results achieved. The report concludes with a statement of the financial position and a list of the members of the committee.

The second part of the report deals with the various projects and schemes undertaken during the year. It is followed by a detailed account of the results achieved and a summary of the progress made. The report concludes with a statement of the financial position and a list of the members of the committee.

The third part of the report deals with the various projects and schemes undertaken during the year. It is followed by a detailed account of the results achieved and a summary of the progress made. The report concludes with a statement of the financial position and a list of the members of the committee.

SUMMARY

1. The effect of physical, nutritional and biological factors on the fruiting and to some extent on other phases of development are reported in the present study.
2. The association of two colonies of the same or different strains of O. graminis either in artificial culture or on the host did not induce perithecial formation.
3. Colonies of different strains were moderately growth-antagonistic, a phenomenon not exhibited by colonies of the same strain.
4. Single strains sporulated on several occasions on wheat seedlings, supporting the view of other workers that O. graminis is homothallic.
5. Although highly pathogenic strains tended to be more fertile than less pathogenic strains, one highly pathogenic isolate exhibited only slight sporulating ability.
6. Certain strains were still fertile after growing in pure culture for several years.
7. Two strains while growing on agar produced spermatogonia-like bodies which at times contained tiny spore structures

SECRET

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similar to spermatia. Other strains produced black perithecia-like bodies under certain nutritional conditions.

8. A method of inducing the formation of perithecia on the host plant was developed and used repeatedly with success. This consisted of growing wheat seedlings in small flasks containing soil artificially infested with O. graminis. Three methods suggested by other workers were unsuccessful when tested.

9. Mature perithecia developed on the host only under conditions of natural daylight, high humidity and relatively cool temperatures (15°C). These physical conditions were maintained throughout most of the studies reported here.

10. Fruiting did not occur in agar cultures exposed to outdoor environment for several weeks.

11. Light was found to be necessary for the maturation of perithecia. Time of exposure had no effect on the number of fruiting bodies formed or on ascospore length.

12. The organism failed to fruit on artificially-inoculated wheat plants growing outside in soil having a high moisture content.

13. Ninety-eight microorganisms, including 59 bacteria,

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes the need for transparency and accountability in financial reporting.

2. The second part of the document outlines the various methods and techniques used to collect and analyze data. It includes a detailed description of the experimental procedures and the statistical tools employed.

3. The third part of the document presents the results of the study, showing the trends and patterns observed in the data. It includes several tables and graphs to illustrate the findings.

4. The fourth part of the document discusses the implications of the results and provides recommendations for future research. It highlights the areas that need further exploration and the potential applications of the findings.

5. The fifth part of the document concludes the study, summarizing the key points and the overall contribution of the research. It expresses the hope that the findings will be useful to other researchers and practitioners in the field.

24 actinomycetes, and 14 fungi failed to stimulate fruiting of O. graminis when associated with the latter in artificial culture on agar.

14. Eight microorganisms added to potato dextrose agar cultures of O. graminis did not induce fruiting when planted 0, 5 and 10 days after O. graminis. A Trichoderma sp. was also ineffective at 5, 10 and 15 days and overgrew O. graminis at all three dates. There was a great difference in the growth reactions between plantings made at different dates.

15. Eight of the above organisms did not stimulate fruiting of O. graminis when introduced singly into sterile flasks containing diseased wheat seedlings growing under relatively sterile conditions.

16. One bacterium (012) appeared to appreciably increase perithecial formation.

17. One set of sterile flasks after contamination with natural soil bore fewer perithecia than sterile checks.

18. Artificially-infected wheat seedlings did not develop perithecia in the greenhouse when planted in association with unsterile wheat stubble or when grown in sterile soil watered with a suspension of natural soil.

19. The necessity of biotin for growth and of thiamin for further vegetative development of O. graminis was confirmed. A concentration of 100 µg. of biotin and 10,000 µg. of thiamin per litre of basic nutrient solution proved optimum for mycelial development.

20. In these studies high concentrations of biotin inhibited growth of O. graminis, a phenomenon not previously reported.

21. It was shown that aspartic acid could be substituted for biotin as a growth factor for O. graminis.

22. The addition of niacin, riboflavin, calcium pantothenate, pyridoxine, folic acid, p-amino benzoic acid, inositol, and vitamin C, singly and in combination, to the basic nutrient solution containing biotin and thiamin in optimum concentrations had no obvious effect on growth.

23. The diluting of nutrients while holding vitamin concentration constant greatly reduced the amount of growth.

24. Of several carbohydrates tested, maltose was the best source for growth and inulin the poorest. Dextrose at the rate of 2% was optimum for mycelial development but concentrations over a wide range were satisfactory.

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25. O. graminis utilized certain nitrogen sources for growth much better than others. Peptone and asparagine were the best sources.
26. D-glutamic acid was more effective than 12 other amino acids supplied singly or in a mixture. NaNO_3 induced induced more growth than several of these amino acids.
27. Growth was not affected by change (before autoclaving) of pH from 3.1 - 9.0 when the organism was cultured on potato dextrose agar.
28. In all of the nutritional studies in pure culture, mature perithecia did not develop although cultures were incubated for 8 weeks under conditions of high humidity and relatively cool temperature in the natural light of the greenhouse.
29. O. graminis is specific in its nutritional requirements for the production of perithecia-like black bodies. Biotin and thiamin are both required; no bodies developed below a concentration of 1.0 μg . of biotin per litre. Pyridoxine, folic acid and p-amino benzoic acid seemed to increase their production slightly.
30. Perithecia-like bodies did not develop in agar culture when a high vitamin-nutrient ratio was maintained.

31. When growth factors were supplied as wheat-stem extract, a 5% concentration of dextrose was required for black body formation; but 2% was effective when biotin and thiamin were supplied in a highly purified form.

32. Of 17 nitrogen sources tested, only NaNO_3 and dl-serine induced the production of perithecia-like bodies.

33. In general, these studies indicate that although several factors influence the degree of fruiting of O. graminis a proper balance between these factors is probably required for the initiation of fruiting.

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